CLINICAL EPIDEMIOLOGY AND DIAGNOSIS OF AMPHOTERICIN B-INDUCED ACUTE KIDNEY INJURY

A Thesis

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ABSTRACT

MANUSCRIPT 1: INCIDENCE, PREDICTORS AND IMPACT OF AMPHOTERICIN B NEPHROTOXICITY ON HOSPITAL MORTALITY USING NEWER ACUTE KIDNEY INJURY DIAGNOSTIC CRITERIA: Studies on amphotericin B (AmB) nephrotoxicity use diverse definitions of acute kidney injury (AKI). Herein, we used the new KDIGO system to describe the incidence, predictors and impact of AmB-induced AKI on hospital mortality in 162 patients treated with AmB (120 deoxycholate and 42 liposomal). KDIGO stage 1 requires an absolute increase ≥0.3 mg/dl or ≥1.5x over baseline serum creatinine (SCr); stage 2 ≥2x, and stage 3 ≥3x. A binary KDIGO definition (KDIGObin) corresponds to stage ≥ 1. For comparison, we included two definitions of AKI traditionally utilized in nephrotoxicity studies: ≥0.5 mg/dl (NT0.5) and ≥2x (NT2x) increase in baseline SCr. The overall incidence of AmB-induced AKI by KDIGObin was 58.6% (staged as: 1=30.9%; 2=18.5% and, 3=9.3%). Predictors of AKI by KDIGObin were older age and use of furosemide and ACE-I. Traditional criteria detected lower incidences of AKI: 45.1% (NT0.5) and 27.8% (NT2x). Predictors of AKI by traditional criteria were older age and use of vancomycin (NT0.5) and use of vancomycin and vasopressors (NT2x). KDIGObin detected AKI 2 days earlier than the most sensitive traditional criteria. However, only traditional criteria were associated with ICU admission, mechanical ventilation and mortality. In conclusion, the increase in sensitivity of KDIGObin is accompanied by a loss of specificity and ability to predict outcomes. Prospective studies are required to weigh the potential gain

from early AKI detection against the potential loss from undue changes in management in patients with subtle elevations in SCr.

MANUSCRIPT 2: ROLE OF URINE NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN IN THE EARLY DIAGNOSIS OF AMPHOTERICIN B-INDUCED ACUTE KIDNEY INJURY: Neutrophil Gelatinase-Associated Lipocalin (NGAL) detects acute kidney injury (AKI) earlier than serum creatinine (SCr) in cardiac surgery, contrast nephropathy and intensive care units. We hypothesized that urine NGAL (UrNGAL) would be an early biomarker of drug nephrotoxicity. We studied hemodynamically stable patients treated with Amphotericin B (AmB). We measured SCr and UrNGAL at baseline and daily after initiation of AmB up to day 14 or development of AKI by SCr criteria. AKI was defined according to Kidney Diseases Improving Global Outcomes (KDIGO) criteria (increase in SCr by ≥0.3 mg/dl within 48 hours or ≥1.5x baseline within 7 days). We studied 24 patients with a mean age of 48.4±16.4 years. Most were male and received AmB (12 deoxycholate and 12 liposomal) for the treatment of leishmaniasis (91.7%). Overall, 17/24 patients fullfilled KDIGO criteria for AKI. Peak UrNGAL levels were higher in AKI than in No AKI patients and in recipients of deoxycholate than liposomal AmB. The diagnostic performance of UrNGAL on day 5 to detect AKI was moderate, with an AUC 0.68 (95% CI 0.41-0.95). In the deoxycholate subgroup, however, the AUC rose to 0.89 (95% CI 0.67- 1.00). In a patient-level analysis, we found that UrNGAL was able to detect AKI 3.2 days earlier than SCr (3.7±2.5 vs. 6.9±3.3 days, time to AKI by UrNGAL and SCr criteria, respectively; p=0.001). Future studies should evaluate if a UrNGAL-oriented treatment strategy will improve outcomes.

BIOGRAPHICAL SKETCH

Paulo Novis Rocha graduated from the Medical School of Bahia of the Federal University of Bahia in 1995. He was an intern, resident and chief resident in Internal Medicine at the Medical College of Pennsylvania between 1996 and 2000. Dr. Rocha was inducted to Alpha Omega Alpha Honor Medical Society in 2000. His fellowship in Nephrology took place at Duke University Medical Center (2000 – 2003), where he trained under Dr. Thomas Myron Coffman. He is board certified in Internal Medicine and Nephrology by the American Boards of Internal Medicine. For the work produced during his fellowship training, he was awarded a Young Investigator Award by Amgen (2002) and a Poster of Distinction award at the American Transplant Congress (2004). After moving back to his home country of Brazil, Dr. Rocha received a PhD degree from the Federal University of Bahia in 2005. In 2007, he was admitted to the Medical School of Bahia of the Federal University of Bahia as an Associate Professor of the Department of Internal Medicine. Since then, he has received honors from seven graduating classes of medical students for excellence in teaching. Dr. Rocha has published 32 articles, 23 of them indexed in Medline. His H-index is 10 by Web of Sciences and 12 by Google Scholar. His main research interests are acute kidney injury, acid-base and electrolyte disorders and immunology of transplants.

This work is dedicated to my father, Heonir Rocha.

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Title: Incidence, Predictors and Impact of Amphotericin B Nephrotoxicity on Hospital Mortality using Newer AKI Diagnostic Criteria

Running title: Amphotericin B-induced Acute Kidney Injury

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ABSTRACT

Studies on amphotericin B (AmB) nephrotoxicity use diverse definitions of acute kidney injury (AKI). Herein, we used the new KDIGO system to describe the incidence, predictors and impact of AmB-induced AKI on hospital mortality in 162 patients treated with AmB (120 deoxycholate and 42 liposomal). KDIGO stage 1 requires an absolute increase ≥ 0.3 mg/dl or ≥ 1.5x over baseline serum creatinine (SCr); stage 2 ≥ 2x, and stage 3 ≥ 3x. A binary KDIGO definition (KDIGObin) corresponds to stage ≥ 1. For comparison, we included two definitions of AKI traditionally utilized in nephrotoxicity studies: ≥ 0.5 mg/dl (NT0.5) and ≥ 2x (NT2x) increase in baseline SCr. The overall incidence of AmB-induced AKI by KDIGObin was 58.6% (staged as: 1 = 30.9%; 2 = 18.5% and, 3 = 9.3%). Predictors of AKI by KDIGObin were older age and use of furosemide and ACE-I. Traditional criteria detected lower incidences of AKI: 45.1% (NT0.5) and 27.8% (NT2x). Predictors of AKI by traditional criteria were older age and use of vancomycin (NT0.5) and use of vancomycin and vasopressors (NT2x). KDIGObin detected AKI 2 days earlier than the most sensitive traditional criteria. However, only traditional criteria were associated with ICU admission, mechanical ventilation and mortality. In conclusion, the increase in sensitivity of KDIGObin is accompanied by a loss of specificity and ability to predict outcomes. Prospective studies are required to weigh the potential gain from early AKI detection against the potential loss from undue changes in management in patients with subtle elevations in SCr.

INTRODUCTION

Amphotericin B (AmB) is a powerful antifungal, antiparasitic agent that binds to the ergosterol component of microbial membranes, creating pores that result in cation leakage and cell death (1). AmB is the drug of choice for the treatment of severe forms of leishmaniasis and remains a lifesaving option for certain invasive fungal infections. Nevertheless, its use is limited by toxicity, including acute kidney injury (AKI).

AmB administration leads to direct renal vasoconstriction and causes a profound reduction in renal blood flow (2–4). In addition, AmB alters renal tubular cell membrane permeability (5, 6), allowing back diffusion of hydrogen ion and thereby impairing acid excretion. Recent data suggests that sodium entry through membrane pores activates MAP kinases and increases intracellular calcium concentration, culminating in renal tubular cell injury (7). Therefore, AmB-induced AKI appears to result from a combination of ischemic and toxic insults (8). Phenotypically, AmB-induced AKI manifests itself through elevation in serum creatinine (SCr) that may be accompanied by a renal tubular acidosis, characterized by hyperchloremic metabolic acidosis, hypokalemia and hypomagnesemia.

Mistro and coworkers systematically reviewed the literature on AmB-induced AKI and assessed whether drug delivery in a locally prepared lipid emulsion or in liposomes reduced nephrotoxicity (9). In their metanalysis, the authors summarized nine clinical trials comparing AmB in 5% dextrose with AmB in lipid emulsion and found an overall incidence of nephrotoxicity in 30,6% versus 12,2%, respectively. They also summarized five clinical trials

comparing AmB in 5% dextrose with liposomal AmB; the incidences of nephrotoxicity were 32,5% versus 14,5%, respectively. Nevertheless, a closer look at individual clinical trials included in the metanalysis uncovers several issues. Most studies were small (only four had more than 100 patients); the populations were quite different and; the incidences of AmB-induced nephrotoxicity varied widely, ranging from 1.3 % (10) to 100% (11) for AmB in 5% dextrose, 1.2% (10) to 33.3% (12) for AmB in lipid emulsion and 0% (13) and 18.7% for liposomal AmB (14). Most importantly, the definition of nephrotoxicity across trials was inconsistent. Since the trials included in the metanalysis by Mistro et al. were published more than 13 years ago (all between 1992 and 2002), none of them used currently accepted consensus definitions of AKI (15–17). Several different AKI criteria were used, but the most common were a ≥ 0.5 mg/dl increase in baseline serum creatinine or ≥ 50% decrease in estimated glomerular filtration rate (GFR) (18) and a doubling of baseline SCr (13, 14, 19, 20).

The first consensus criteria for AKI was published in 2004 and called RIFLE (Risk, Injury, Failure, End stage) (15). In 2007, the RIFLE criteria was modified to create the AKIN (Acute Kidney Injury Network) criteria (16). Finally, in 2012, characteristics of both RIFLE and AKIN were merged to create the KDIGO (Kidney Disease Improving Global Outcome) criteria (17). The KDIGO system uses changes in SCr or urine output to diagnose and stratify AKI in three stages. Minimal changes in SCr, such as an increase ≥ 1.5x over baseline or an abrupt (over a 48-hour) increase in ≥ 0.3 mg/dl would qualify as AKI by KDIGO stage 1. A doubling of baseline SCr, which, as mentioned above, is the

criterion most commonly used to define AmB-induced AKI in older studies, would qualify as KDIGO stage 2. A tripling of baseline SCr or need for dialysis is defined as KDIGO stage 3.

Minejima et al. (21) demonstrated that the AKIN criteria facilitated the early detection of vancomycin-induced nephrotoxicity when compared to traditional nephrotoxicity criteria (NT, defined as a ≥ 0.5 mg/dl increase in baseline SCr or a ≥ 50% decrease in estimated GFR). To our knowledge, there are no studies comparing the performance of newer versus traditional diagnostic criteria on AmB-induced AKI.

The main objective of this study was to identify the incidence and predictors of AmB-induced AKI according to currently accepted staging systems. In addition, we aimed to describe the dynamics of AmB-induced AKI and assess its impact on hospital mortality.

MATERIALS AND METHODS

Site: The study was conducted at Hospital Universitário Professor Edgard Santos, a tertiary care facility affiliated with the Medical School of the Federal University of Bahia, located in Salvador, Bahia, Brazil.

Design and population: This was a retrospective cohort study of inpatients treated with AmB between 2006 and 2012. The year 2006 was chosen because it was the first year after the introduction of electronic medical records (EMRs) for laboratory data at our institution (August 2005); the year 2012 was the date of the initial draft of this project. According to our inpatient pharmacy, there were 734 treatments with AmB during that period of time.

Exclusion criteria were re-treatments (in these cases, only data from the first treatment was analyzed); use of less than three doses of AmB; age less than 18 years old; AKI or hemodialysis at the time of AmB initiation; intermittent use of AmB, such as weekly use in "day hospital" setting; use of non-intravenous forms of AmB and; missing critical data.

Variables collected: We reviewed paper charts and electronic medical records for demographic variables, patient location (floor versus ICU), reason for AmB use, type of AmB used, AmB regimen, total AmB dose, renal protection strategy used, renal consultation (timing, recommendations), comorbidities (Charlson comorbidity index), concomitant nephrotoxic medications, use and dose of vasoactive drugs, serial laboratory data (BUN, SCr, potassium, magnesium and bicarbonate), days on the ventilator (if applicable), days in the ICU (if applicable), days in the hospital, and in-hospital mortality.

Definitions: AKI was defined and staged according to RIFLE (15), AKIN (16) and KDIGO (17) criteria. For comparison, we also defined AKI using two traditional nephrotoxicity (NT) criteria (21). Since we did not have access to urinary output data, AKI diagnoses were based solely on the magnitude of the increases in SCr:

- NT0.5: absolute increase in SCr ≥ 0.5 mg/dl over baseline (no additional staging system);
- NT2x: increase in SCr ≥ 2x over baseline (no additional staging system);
- RIFLE R: increase in SCr ≥ 1.5x over baseline; RIFLE I: increase in SCr ≥ 2x over baseline; RIFLE F: increase in SCr ≥ 3x over baseline.

- AKIN stage I: absolute increase in SCr ≥ 0.3 mg/dl over baseline within 48 hours; stage II: increase in SCr ≥ 2x over baseline; stage III: increase in SCr to ≥ 3x over baseline or initiation of dialysis;

- KDIGO stage 1: absolute increase in SCr ≥ 0.3 mg/dl over baseline within 48 hours *or* increase in SCr ≥ 1.5x over baseline; stage 2: ≥ increase in SCr 2x over baseline; stage 3: increase in SCr ≥ 3x over baseline or initiation of dialysis.

Due to the 48-hour requirement, which practically entails having daily SCr measurements (or at least every other day), the AKIN criteria could not be applied to some patients. Since the newest KDIGO system combines characteristics of RIFLE and AKIN criteria, we chose this system to stratify AKI in ordinal stages. In addition, we used binary definitions for RIFLE, AKIN and KDIGO (RIFLEbin, AKINbin and KDIGObin). In these binary definitions, any patient that fulfilled criteria for the initial stage (which in practice means a stage R or greater for RIFLE, stage I or grater for AKIN or stage 1 or greater for KDIGO) was classified as having AKI.

The baseline SCr was defined as the value obtained on the morning of day zero, which was the day of the first AmB dose. If a SCr value was not available for that day, we used the value nearest to day zero or the admission SCr.

Hypocalemia and hypomagnesemia were diagnosed when at least one of three conditions was satisfied: 1) the presence of documented K+ or Mg++ levels below the laboratory's limit of normal during AmB treatment; 2) a history of replacement of these cations; or, 3) when these electrolyte disorders were listed as problems in the patient's chart.

Ethical approval: The study was approved by the institutional review board of Hospital Universitário Professor Edgard Santos on 03/11/13 (protocol number 11123413.1.0000.0049). Given the retrospective nature of the study, we were granted a waiver of informed consent.

Statistical analyses: The shape of the distribution of continuous data was analyzed using histograms and normality tests (Shapiro Wilk). Normal-shaped data were summarized using mean and standard deviation and comparisons among groups made with the Student's t test; non-Gaussian data were summarized using median and interquartile range and comparisons among groups made with the Mann Whitney U test. Categorical data were summarized using absolute and relative frequencies and comparisons among groups made with the chi square or Fisher's exact test, where appropriate. Time to AKI was analyzed in two different ways: 1) by summarizing the mean and median time to AKI only in those who developed AKI; 2) by the Kaplan Meier method. For those patients whose lengths of follow up exceeded one month, the survival time was censored at thirty days. The Kaplan Meier data was summarized in one minus cumulative survival graphs and survival curves compared using the log-rank test. Univariate logistic and Cox regression analyses were conducted to identify potential predictors of AKI. Variables with a p value < 0.20 on univariate analyses were included in multivariate, backward, logistic and Cox regression models to identify independent predictors of AKI. A similar strategy was utilized to identify independent predictors of death. However, since AKI was our main independent variable in time to mortality analyses, it was allocated to the second block of the

regression and forced into the final model. A p value < 0.05 on final analyses was considered statistically significant. Statistical analyses were conducted using the software packages STATA version 12.1 and IBM SPSS Statistics version 20.0.

Sample size: A priori sample size calculation was conducted using Open EPi, available at http://openepi.com/OE2.3/Menu/OpenEpiMenu.htm. Assuming an overall nephrotoxicity rate of 30% and a precision of 5%, a sample size of 122 would provide a confidence level of approximately 80%; a sample size of 186 patients, 90%; and a sample size of 245 patients would provide a confidence level of 95%.

RESULTS

Study population

Out of 734 treatments with AmB that occurred at our institution between 2006 and 2012, 72 consisted of re-treatments and were excluded. Out of the 662 patients that used AmB, 500 were excluded. Our final sample was comprised of a cohort of 162 patients treated with AmB, 120 with the deoxycholate 42 with the liposomal preparation (Figure 1.1). The median length of stay in the hospital for the cohort was 32 days (interquartile range, IQR 23 to 51 days).

Table 1.1 shows the demographic, clinical and laboratory characteristics of the final sample. Patients were young, with a median age of 36, and there was a slight preponderance of males. The main indications for AmB were suspected or confirmed fungal infection (40.8%), leishmaniasis (27.8%) and fever of

unknown origin (22.2%). Only 23.5% had a Charlson comorbidity index ≥ 4; approximately 1/3 needed to be treated at the intensive care unit at some point during the admission. Median baseline laboratory values were within normal limits.

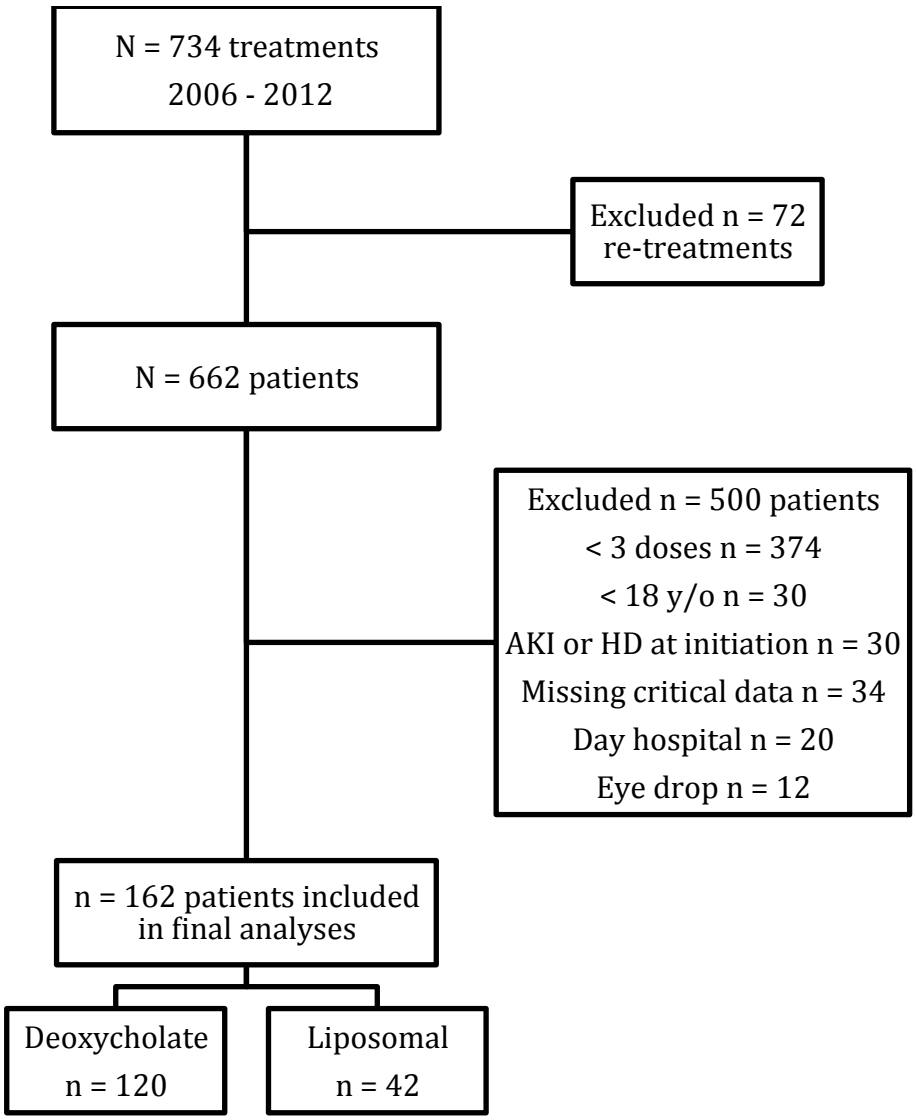

Figure 1.1. Flow chart demonstrating the process of selection of the sample of 162 patients treated with amphotericin B at our institution between 2006 and 2012. Legend: AKI = acute kidney injury; HD = hemodialysis

Table 1.1. Demographic, clinical and laboratory characteristics of 162 hospitalized adults treated with intravenous AmB at a university hospital.

	N = 162
Age, years (median, IQR), n = 152	36 [26 to 51]
Gender	
Male	92/162 (56.8%)
Female	70/162 (43.2%)
Place of residence	
Rural areas of the state	95/160 (59.4%)
Capital	65/160 (40.6%)
Indication for AmB	
Tegumentary leishmaniasis	33/162 (20.4%)
Visceral leishmaniasis	12/162 (7.4%)
Suspected invasive fungal infection	26/162 (16.0%)
Confirmed invasive fungal infection	40/162 (24.8%)
Fever of unknown origin	36/162 (22.2%)
Other	15/162 (9.3%)
Charlson comorbidity index	
0-1	65/162 (40.1%)
2-3	59/162 (36.4%)
4 or greater	38/162 (23.5%)
Other variables related to disease severity	
Intensive care at some point	53/162 (32.7%)
Vasopressor drugs at some point	35/162 (21.6%)
Mechanical ventilation	42/162 (26.1%)
Baseline laboratory values (serum)	
Creatinine, mg/dl (n = 162)	0.8 [0.7 to 1.1]
BUN, mg/dl (n = 103)	12.2 [7.9 to 18.2]
Potassium, meq/l (n = 98)	4.0 [3.6 to 4.4]
Magnesium, mg/dl (n = 73)	1.8 [1.6 to 2.0]
Bicarbonate, meq/l (n = 28)	21.6 [16.7 to 26.3]
Lactate, mmol/l (n = 23)	1.6 [1.2 to 2.0]

Legend: AmB = amphotericin B; BUN = blood urea nitrogen. Continuous data are presented as median [25th percentile to 75th percentile].

Treatment details

The majority (83.3%) of patients initiated AmB at the ward. The deoxycholate preparation was used by 74.1% of patients; the median initial and total doses were 50 mg and 445 mg, respectively. The liposomal preparation was used by 25.9% of patients; the median initial and total doses were 150 mg and 1400 mg, respectively. Median duration of AmB treatment was 10 days. The median dose of normal saline used at the beginning of AmB treatment was 1500 cc/day. The nephrotoxic drug that was most commonly used in combination with AmB was vancomycin (40.1% of cases).

Incidence of AmB-induced AKI

The overall incidence of AmB-induced AKI by traditional criteria was 27.8% (45/162) for NT2x and 45.1% (73/162) for NT0.5 (Table 1.2). The RIFLE criteria diagnosed 10 additional cases of AKI, for an incidence of 51.2% (83/162). The KDIGO criteria was the most sensitive, detecting 22 cases that were missed by the NT0.5 criteria, for an AKI incidence of 58.6% (95/162).

The AKIN criteria could not be applied in 25 cases due to the absence of SCr values at critical time points, which hampered our ability to comply with the 48-hour requirement. Since the KDIGO criteria combines characteristics of RIFLE and AKIN, this system was chosen to stratify AKI in stages. Most cases of AKI were mild, classified as stage 1 or 2. The incidence of severe, KDIGO stage 3-AKI was less than 10% overall and less than 5% in patients using the liposomal preparation.

Table 1.2. Influence of the diagnostic criteria on the incidence of AKI during intravenous AmB use in 162 hospitalized adults treated at a university hospital, stratified by type of AmB preparation.

AKI criteria	All (n = 162)	Type of AmB		p
		Deoxycholate (n = 120)	Liposomal (n = 42)	
Binary				
NT2x	45/162 (27.8%)	38/120 (31.7%)	7/42 (16.7%)	0.062
NT0.5	73/162 (45.1%)	59/120 (49.2%)	14/42 (33.3%)	0.076
RIFLEbin	83/162 (51.2%)	65/120 (54.2%)	18/42 (42.9%)	0.207
AKINbin*	72/137 (52.6%)	55/102 (53.9%)	17/35 (48.6%)	0.584
KDIGObin	95/162 (58.6%)	72/120 (60.0%)	23/42 (54.8%)	0.553
KDIGO stages#				
Stage 1	50/162 (30.9%)	34/120 (28.3%)	16/42 (38.1%)	
Stage 2	30/162 (18.5%)	25/120 (20.8%)	5/42 (11.9%)	0.290
Stage 3	15/162 (9.3%)	13/120 (10.8%)	2/42 (4.8%)	

Legend: NT2x = traditional nephrotoxicity criteria of a serum creatinine increase ≥ 2x over baseline; NT0.5 = traditional nephrotoxicity criteria of an absolute serum creatinine increase ≥ 0.5 mg/dl over baseline; RIFLE = Risk, Injury, Failure, Loss, End-stage; a RIFLEbin indicates a serum creatinine increase ≥ 1.5x over baseline; AKIN = Acute Kidney Injury Network; an AKINbin indicates an absolute serum creatinine increase ≥ 0.3 mg/dl over baseline; KDIGO = Kidney Diseases Improving Global Outcomes; a KDIGObin indicates an absolute serum creatinine increase ≥ 0.3 mg/dl or ≥ 1.5x over baseline. *Unable to use AKIN criteria in 25 patients due to the 48-hour requirement. #Numbers represent the maximum stage. Some patients with KDIGO stages 2 or 3 passed thru stage 1, but they were classified as the maximum stage that they reached.

The incidence of AKI was numerically higher in the deoxycholate than in the liposomal group, but the differences were only marginally significant when using traditional NT0.5 or NT2x criteria. Patients on liposomal AmB also tended to have less severe cases of AKI, with a lower percentage of cases classified as stages 2 or 3.

Regarding the time to diagnosis of AKI, the traditional NT0.5 criteria detected AmB-induced AKI at a median of 6.0 days [IQR 4.0 to 9.0] whereas the newer KDIGO criteria detected it two days earlier, at a median of 4.0 days [IQR 2.0 to 7.8] (Table 1.3).

Table 1.3. Influence of the diagnostic criteria on the time in days to diagnosis of AKI during intravenous AmB use in 162 hospitalized adults treated at a university hospital, stratified by type of AmB preparation.

AKI Criteria	All (n = 162)	Type of AmB	
		Deoxycholate (n = 120)	Liposomal (n = 42)
NT0.5 (n = 73)	6.0 [4.0 to 9.0]	5.0 [4.0 to 9.0]	7.0 [4.0 to 12.5]
NT2x (n=45)	5.0 [3.0 to 8.0]	5.0 [3.0 to 980]	6.0 [4.0 to 8.0]
KDIGO			
Stage 1 (n = 76)	4.0 [2.0 to 7.8]	4.0 [2.5 to 7.5]	4.0 [2.0 to 8.0]
Stage 2 (n = 40)	5.5 [3.3 to 8.0]	5.5 [3.0 to 8.3]	5.5 [3.5 to 8.8]
Stage 3 (n = 15)	7.0 [4.0 to 13.0]	7.0 [3.5 to 13.0]	8.0 [6.0 to 10.0]

Legend: NT2x = traditional nephrotoxicity criteria of a serum creatinine increase ≥ 2x over baseline; NT0.5 = traditional nephrotoxicity criteria of an absolute serum creatinine increase ≥ 0.5 mg/dl over baseline; KDIGO = Kidney Diseases Improving Global Outcomes. The number of patients with AKI by the KDIGO criteria was 95. The sum of patients across the KDIGO stages add up to more than 95 because some patients evolved from stage 1 to stages 2 and 3 during treatment.

In a survival analysis using the Kaplan Meier method, the KDIGObin also detected more cases of AKI - and at earlier time points - than the traditional NT0.5 criteria (Figure 1.2). Nevertheless, this increased sensitivity of the KDIGObin definition occurred at the expense of a decreased ability to discriminate the differential nephrotoxic profiles of the two AmB preparations.

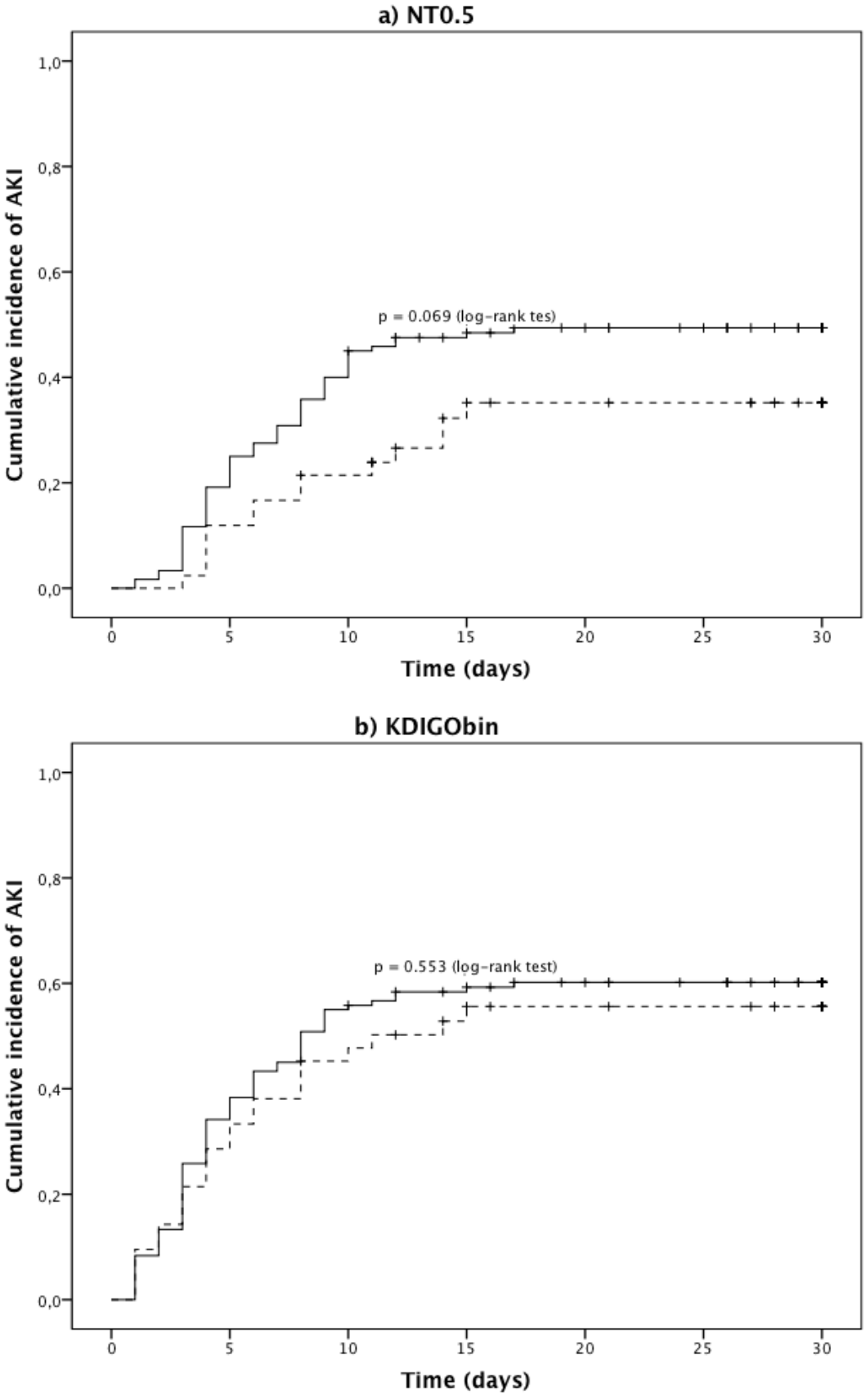

Figure 1.2. Time to AKI stratified by AmB preparation according to NT0.5 and KDIGObin criteria. Legend: NT0.5 = traditional nephrotoxicity criteria of

an absolute serum creatinine increase ≥ 0.5 mg/dl over baseline; NT2x = traditional nephrotoxicity criteria of a serum creatinine increase ≥ 2x over baseline. KDIGO = Kidney Diseases Improving Global Outcomes; a KDIGObin indicates an absolute serum creatinine increase ≥ 0.3 mg/dl or ≥ 1.5x over baseline. Solid line: deoxycholate; Dashed line: liposomal. (| and +) marks on the survival curves represent censored cases. Comparison among one minus cumulative survival curves for the two AmB preparations was 0.069 using the NT0.5 criteria, 0.081 using NT2x (not shown) and 0.553 using the KDIGObin criteria (log-rank test).

Clinical characteristics of AmB-induced AKI

Figure 1.3 shows mean serum creatinine values during AmB therapy, stratified by the presence of AKI by KDIGObin. In the AKI group, mean serum creatinine started to rise at day two and peaked at day 8. Peak serum creatinine in the AKI group was (mean ± SD) 1.66 ± 0.67 mg/dl versus 1.04 ± 0.38 mg/dl in the no AKI group (p = <0.001). Peak serum creatinine was also numerically higher among AKI patients using deoxycholate versus liposomal preparation (1.69 ± 0.71 mg/dl versus 1.58 ± 0.53 mg/dl), but the difference was not statistically significant (p = 0.488).

Electrolyte imbalances

Regarding electrolyte imbalances during AmB treatment, 121/162 (74.7%) fulfilled our criteria for hypokalemia and 107/162 (66.0%) required potassium replacement. Both hypokalemia (80.0% versus 59.5%, p = 0.015) and potassium replacement (71.7% versus 50.0%, p = 0.018) were significantly more common in patients using the deoxycholate versus the liposomal preparation.

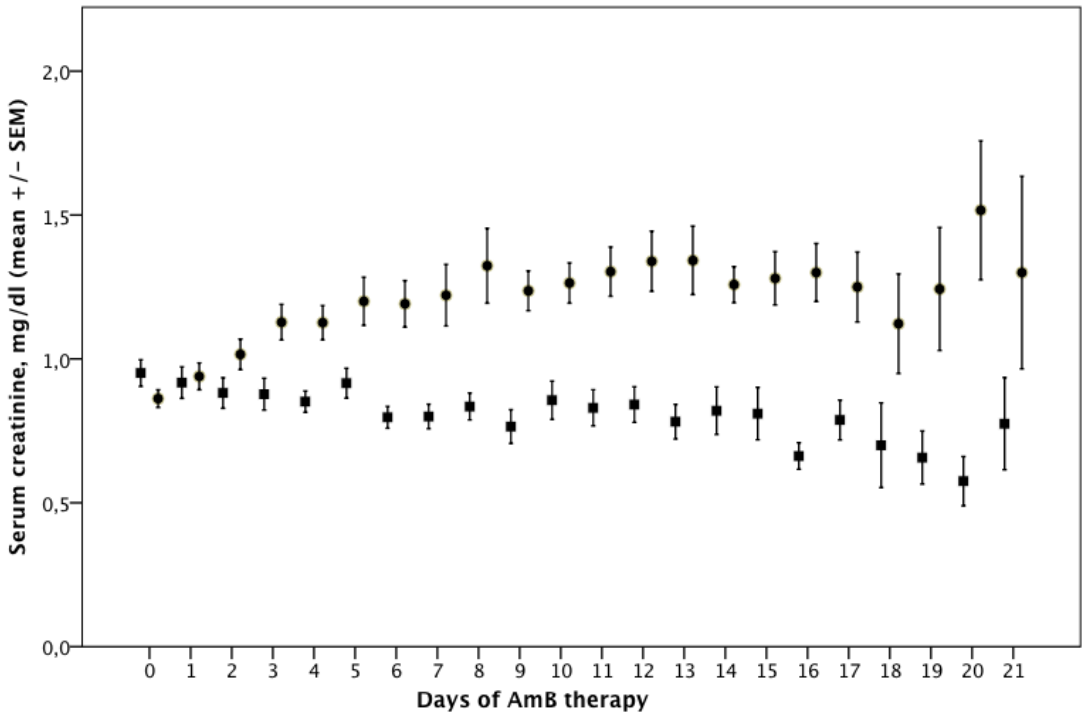

Figure 1.3. Serum creatinines over time during AmB use, stratified by the occurrence of AKI according to the KDIGO criteria. Legend: the circles represent the AKI group and squares represent those without AKI by the KDIGO (Kidney Diseases Improving Global Outcomes) criteria. A KDIGObin indicates an absolute serum creatinine increase ≥ 0.3 mg/dl or ≥ 1.5x over baseline.

Hypomagnesemia was detected in 101/162 (62.3%) and magnesium replacement was required by 89/162 (54.9%) of patients. Similarly, hypomagnesemia (68.3% versus 45.2%, p = 0.10) and magnesium replacement (60.8% versus 38.1%, p = 0.012) were more common in patients receiving deoxycholate versus liposomal AmB.

There was no association, however, between these electrolyte imbalances and AKI, regardless of the AKI criteria.

AKI management and Nephrology consult

A change in management in response to AKI was documented in 59/95 (62.1%) patients classified by KDIGObin. Changes in management were more frequent in stage 3 (73%) than in stages 2 (66.7%) or stage 1 (56%) AKI. The most common changes were to discontinue AmB (n=32); increase the amount of intravenous fluids (n=19); decrease AmB dose (n=17); switch from deoxycholate to liposomal (n=8); switch the AmB regimen (e.g. from daily to every other day, n = 6), and; discontinue concomitant nephrotoxins (n=3).

A Nephrology consult was performed in 26.3% (25/95) of patients with and in 6% (4/67) of patients without AKI by KDIGObin. There was a significant association between nephrology consult and KDIGO stage, with higher frequency of consultation occurring at higher stages (9.6% at stage 1, 25% at stage 2 and 86.7% at stage 3, p = <0.001). The median time from initiation of AmB treatment to nephrology consult was 5 days (IQR 1.0 to 8.5). Median serum creatinine at consultation was 1.4 mg/dl (IQR 1.2 to 2.3).

Eleven patients (6.8%) required dialysis at a median of 9.0 [IQR 5.0 to 15.0] days after initiation of AmB. Median time on dialysis was 3.0 days [IQR 0.0 to 8.0 days].

Predictors of AmB-induced AKI

We then attempted to identify predictors of AKI. For these analyses, we used both logistic regression (treating AKI as the binary dependent variable) and Cox proportional hazards (using time to AKI as the dependent variable) analyses. In addition, we conducted these analyzes using KDIGObin and traditional NT0.5 and NT2x criteria. The following predictor variables were

analyzed: age (continuous), type of AmB (deoxycholate versus liposomal), place of AmB initiation (ICU versus ward), diagnosis (leismaniasis versus other), Charlson score (≥ 4 versus 1 thru 3) and use of furosemide, ACE-I, polimixin B, vancomycin and vasopressors (all yes versus no).

Independent predictors of AKI in multivariate logistic regression models varied according to the criteria used to define AKI. When the KDIGObin criteria was used, age and use of furosemide were marginally significant. Age and use of vancomycin were predictors of AKI by NT0.5 whereas use of vancomycin and vasopressors predicted AKI by NT2x (Table 1.4). Cox proportional hazards models yielded similar findings (Table 1. 5).

Impact of AmB-induced AKI on hospital morbidity and mortality

Using the KDIGObin definition, AmB-induced AKI was not associated with need for ICU admission, mechanical ventilation or mortality. When looking at the different KDIGO stages, the proportion of patients with KDIGO stage 1 that required ICU, mechanical ventilation or died was actually lower than that of patients without AKI. Traditional NT criteria and KDIGO stages 2 or 3, however, were highly associated with all three outcomes (Table 1.6).

Overall, 45/162 patients (27.8%) died during hospital follow up. The median time to death was 32 [IQR 22.5 to 50.5] days. The impact of AKI on mortality was evaluated on univariate and multivariate analyses.

Table 1.4. Univariate and multivariate logistic regression analyses to identify independent predictors of AKI by KDIGObin, NT0.5 and NT2x.

AKI criteria Predictors	**Univariate OR (95% CI)**	**p**	**Multivariate OR (95% CI)**	**p**
KDIGObin				
Age (years)	1.02 (1.00 - 1.04)	**0.066**	1.02 (1.00 - 1.04)	0.096
Deoxycholate	1.24 (0.61 - 2.52)	0.553		
ICU initiation	1.24 (0.53 - 2.91)	0.618		
Leishmaniasis	1.40 (0.69 - 2.85)	0.353		
Charlson ≥ 4	1.28 (0.61 - 2.71)	0.519		
Furosemide	2.26 (1.01 - 5.07)	**0.047**	2.30 (0.98 - 5.39)	0.056
ACE-I	2.29 (0.91 - 5.77)	**0.080**		
Polimixin B	0.59 (0.22 - 1.63)	0.309		
Vancomycin	1.22 (0.64 - 2.32)	0.540		
Vasopressors	1.07 (0.50 - 2.30)	0.854		
NT0.5				
Age (years)	1.02 (1.00 - 1.04)	**0.064**	1.02 (1.00 - 1.04)	0.038
Deoxycholate	1.93 (0.93 - 4.03)	**0.078**		
ICU initiation	1.99 (0.86 - 4.61)	**0.108**		
Leishmaniasis	0.85 (0.43 - 1.71)	0.653		
Charlson ≥ 4	1.13 (0.55 - 2.34)	0.744		
Furosemide	2.13 (1.01 - 4.49)	**0.048**		
ACE-I	1.66 (0.72 - 3.81)	0.233		
Polimixin B	0.84 (0.30 - 2.32)	0.734		
Vancomycin	2.01 (1.06 - 3.81)	**0.032**	2.24 (1.16 - 4.32)	0.016
Vasopressors	1.61 (0.76 - 3.41)	0.218		
NT2x				
Age (years)	1.00 (0.98 - 1.02)	0.965		
Deoxycholate	2.32 (0.94 - 5.69)	**0.067**		
ICU initiation	2.47 (1.05 - 5.81)	**0.038**		
Leishmaniasis	0.47 (0.20 - 1.10)	**0.082**		
Charlson ≥ 4	1.27 (0.58 - 2.81)	0.550		
Furosemide	1.85 (0.85 - 4.02)	**0.080**		
ACE-I	1.68 (0.70 - 4.02)	0.243		
Polimixin B	1.09 (0.36 - 3.30)	0.874		
Vancomycin	2.74 (1.35 - 5.54)	**0.005**	2.27 (1.09 - 4.72)	0.028
Vasopressors	3.34 (1.52 - 7.32)	**0.003**	2.73 (1.21 - 6.16)	0.015

Legend: Age was evaluated as a continuous variable; all other independent variables are categorical. Variables with p <0.20 in univariate analyses (in bold) were included in a multivariate, backward, logistic regression model. Only the variables remaining at the final model are shown.

Table 1.5. Univariate and multivariate Cox regression analyses to identify independent predictors of AKI by KDIGObin, NT0.5 and NT2x.

AKI criteria Predictors	**Univariate** **HR (95% CI)**	**p**	**Multivariate** **HR (95% CI)**	**p**
KDIGObin				
Age (years)	1.09 (1.00 - 1.02)	**0.143**		
Deoxycholate	1.15 (0.72 - 1.83)	0.569		
ICU initiation	1.20 (0.71 - 2.03)	0.497		
Leishmaniasis	1.10 (0.71 - 1.70)	0.679		
Charlson ≥ 4	1.18 (0.37 - 3.77)	0.776		
Furosemide	1.42 (0.91 - 2.21)	**0.127**		
ACE-I	1.52 (0.93 - 2.50)	**0.095**	1.53 (0.92 - 2.53)	0.100
Polimixin B	0.73 (0.35 - 1.50)	0.384		
Vancomycin	1.16 (0.77 - 1.76)	0.466		
Vasopressors	1.10 (0.68 - 1.80)	0.693		
NT0.5				
Age (years)	1.01 (1.00 - 1.02)	**0.104**	1.01 (1.00 - 1.04)	0.084
Deoxycholate	1.68 (0.94 - 3.02)	**0.080**		
ICU initiation	1.59 (0.91 - 2.77)	**0.102**		
Leishmaniasis	0.83 (0.49 - 1.41)	0.833		
Charlson ≥ 4	1.07 (0.63 - 1.83)	0.793		
Furosemide	1.52 (0.92 - 2.51)	**0.101**		
ACE-I	1.29 (0.73 - 2.28)	0.374		
Polimixin B	0.92 (0.42 – 2.00)	0.826		
Vancomycin	1.70 (1.07 - 2.69)	**0.024**	1.76 (1.10 - 2.78)	0.018
Vasopressors	1.45 (0.86 - 2.45)	**0.162**		
NT2x				
Age (years)	1.00 (0.98 - 1.02)	0.889		
Deoxycholate	2.00 (0.89 - 4.48)	**0.092**		
ICU initiation	2.07 (1.17 - 4.01)	**0.031**		
Leishmaniasis	0.51 (0.24 - 1.09)	**0.082**		
Charlson ≥ 4	1.21 (0.63 - 2.34)	0.571		
Furosemide	1.67 (0.89 - 3.14)	**0.112**		
ACE-I	1.50 (0.74 - 3.02)	0.261		
Polimixin B	1.09 (0.43 - 2.77)	0.853		
Vancomycin	2.39 (1.32 - 4.33)	**0.004**	2.00 (1.08 - 3.71)	0.028
Vasopressors	2.58 (1.41 - 4.71)	**0.002**	2.08 (1.11 - 3.91)	0.022

Legend: Age was evaluated as a continuous variable; all other independent variables are categorical. Variables with p <0.20 in univariate analyses (in bold) were included in a multivariate, backward, Cox regression model. Only the variables remaining at the final model are shown.

Table 1.6. Impact of AKI on ICU admission, mechanical ventilation and inpatient mortality.

a) AKI by binary criteria

Outcome AKI criteria	**AKI status**		
ICU admission	**No AKI**	**AKI**	**p**
NT2x	28/117 (23.9%)	24/45 (55.6%)	<0.001
NT0.5	22/89 (24.7%)	31/73% (42.5%)	0.017
KDIGObin	20/67 (29.9%)	33/95 (34.7%)	0.514
Mechanical ventilation	**No AKI**	**AKI**	**p**
NT2x	22/117 (18.8%)	21/45 (46.7%)	<0.001
NT0.5	16/89 (18.0%)	27/73% (37.0%)	0.006
KDIGObin	15/67 (22.4%)	28/95 (29.5%)	0.314
Inpatient mortality	**No AKI**	**AKI**	**p**
NT2x	25/117 (21.4%)	20/45 (44.4%)	0.003
NT0.5	19/89 (21.3%)	26/73 (35.6%)	0.044
KDIGObin	16/67 (23.9%)	29/95 (30.5%)	0.352

b) AKI by KDIGO stages

Variables	**No AKI**	**AKI stages by KDIGO**			**p**
		Stage 1	**Stage 2**	**Stage 3**	
ICU admission	20/67 (29.9%)	8/50 (16.0%)	12/30 (40.0%)	13/15 (86.7%)	<0.001
Mechanical ventilation	15/67 (22.4%)	7/50 (14.0%)	10/30 (33.3%)	11/15 (73.3%)	<0.001
Inpatient mortality	16/67 (23.9%)	9/50 (18.0%)	9/30 (30.0%)	11/15 (73.3%)	<0.001

Legend: NT2x = traditional nephrotoxicity criteria of a serum creatinine increase ≥ 2x over baseline; NT0.5 = traditional nephrotoxicity criteria of an absolute serum creatinine increase ≥ 0.5 mg/dl over baseline; KDIGO = Kidney Diseases Improving Global Outcomes; a KDIGObin indicates an absolute serum creatinine increase ≥ 0.3 mg/dl or ≥ 1.5x over baseline.

On univariate analyses, the NT0.5 criterion was mildly associated with mortality by logistic regression but not by Cox regression (Table 1.7). When AKI was classified using the KDIGO criteria as a binary variable, it became significantly associated with mortality only when stages 2 and 3 (KDIGO ≥ 2) were combined. In fact, when looking at the KDIGO criteria as an ordinal variable, mortality associated with stage 1 AKI (9/52, 17.3%) was even lower than in those without AKI (16/67, 23.9%). Stage 3 AKI and need for dialysis were highly associated with mortality.

Table 1.7. Impact of AKI criteria on the univariate association between AKI and mortality

a) Univariate logistic regression

AKI criteria	n/N (%)	OR (95% CI)	p
Binary			
NT0.5	26/73 (35.6%)	2.04 (1.02 - 4.09)	0.045
NT2x	20/45 (44.4%)	2.94 (1.41 - 6.14)	0.004
KDIGObin	29/95 (30.5%)	1.40 (0.69 - 2.85)	0.353
KDIGO stages			
Stage 1	9/50 (18.0%)	0.70 (0.28 - 1.75)	0.444
Stage 2	9/30 (30.0%)	1.37 (0.52 - 3.57)	0.525
Stage 3	11/15 (73.3%)	8.77 (2.45 - 31.36)	0.001
Dialysis	10/11 (90.9%)	33.14 (4.10 - 267.98)	<0.001

a) Univariate Cox regression

AKI criteria	n/N (%)	HR (95% CI)	p
Binary			
NT0.5	26/73 (35.6%)	1.53 (0.84 - 2.81)	0.168
NT2x	20/45 (44.4%)	2.23 (1.23 - 4.03)	0.008
KDIGObin	29/95 (30.5%)	1.20 (0.65 - 2.24)	0.559
KDIGO stages			
Stage 1	9/50 (18.0%)	0.62 (0.26 - 1.46)	0.270
Stage 2	9/30 (30.0%)	1.19 (0.52 - 2.72)	0.682
Stage 3	11/15 (73.3%)	3.29 (1.52 - 7.13)	0.002
Dialysis	10/11 (90.9%)	5.05 (2.47 - 10.34)	<0.001

Legend: NT2x = traditional nephrotoxicity criteria of a serum creatinine increase ≥ 2x over baseline; NT0.5 = traditional nephrotoxicity criteria of an absolute serum creatinine increase ≥ 0.5 mg/dl over baseline; KDIGO = Kidney Diseases Improving Global Outcomes; a KDIGObin indicates an absolute serum creatinine increase ≥ 0.3 mg/dl or ≥ 1.5x over baseline.

In Table 1.8, we further explored the association between AKI by NT2x with mortality in multivariate models.

Table 1.8. Impact of AKI by NT2x on mortality, adjusted for potential confounders

a) Logistic Regression Model

Model Variables	**OR (95% CI)**	**p**
Unadjusted NT2x	2.94 (1.41 - 6.14)	0.004
Model 1		
NT2x	2.279 (1.007 - 5.16)	0.048
Charlson (≥4 versus 0-3)	2.694 (1.104 - 6.11)	0.033
Steroids	2.960 (1.225 - 7.15)	0.019
Vancomycin	2.715 (1.193 - 6.18)	0.016
Furosemide	2.597 (1.104 - 6.11)	0.029
Model 2		
NT2x	1.06 (0.32 - 3.55)	0.927
Steroids	6.06 (1.59 - 23.02)	0.008
Vasopressors	3.60 (0.99 - 13.09)	0.052
Mechanical ventilation	44.34 (12.28 - 160.02)	<0.001

b) Cox Regression Model

Model Variables	**HR (95% CI)**	**p**
Unadjusted		
NT2x	2.23 (1.23 - 4.03)	0008
Model 1		
NT2x	2.31 (1.27 - 4.19)	0.006
Charlson (≥4 versus 0-3)	2.04 (1.07 - 3.91)	0.031
Steroids	2.12 (1.02 - 4.42)	0.044
Model 2		
NT2x	1.27 (0.68 - 2.35)	0.453
Steroids	2.14 (1.02 - 4.47)	0.044
Vasopressors	2.20 (1.02 - 4.02)	0.045
Mechanical ventilation	5.49 (2.40 - 12.56)	<0.001

Legend: NT2x = traditional nephrotoxicity criteria of a serum creatinine increase ≥ 2x over baseline. Variables included in model 1: First block (backward) Charlson's comorbidity index, use of furosemide, vancomycin and steroids; second block (enter) AKI by NT2x. Variables included in model 2: First block (backward) all variables included in model 1 plus ICU, use of vasopressors and mechanical ventilation; second block (enter) AKI by NT2x.

AKI remained significantly associated with mortality even when adjusted for the Charlson comorbidity index and use of furosemide, vancomycin and steroids (Model 1). However, when use of vasopressors and need for ICU and mechanical ventilation were included (Model 2), AKI no longer predicted death.

None of the 45 patients with a diagnosis of leishmaniasis in our dataset ended up dying during hospital admission. Hence, this variable could not be included as a predictor in multivariate mortality analyses due to mathematical instability of the model. Therefore, we repeated all multivariate logistic and Cox regression analyses for predictors of mortality in the subset of 117 patients with a diagnosis other than leishmaniasis and found similar results (data not shown).

DISCUSSION

In 2001, Bellomo et al (22) called attention to the fact that there were more than 30 different definitions of acute renal failure in use in the medical literature. Their call for a consensus definition of this syndrome was answered in 2004, with the publication of the RIFLE criteria (15). Subsequent evidence that minimal increases in SCr (as small as 0.3 mg/dl) were associated with worse outcomes (23) led to incorporation of smaller increases in SCr into a modification of the RIFLE criteria called AKIN (16). More recently, the KDIGO guidelines attempted to harmonize earlier consensus definitions and staging criteria for AKI (17). Whether these criteria should be applied in routine clinical care is still a matter of debate (24).

Herein, we showed that the overall incidence of AmB-induced AKI by the most sensitive traditional criterion (NT 0.5) was 45.4% and occurred at a median time of 6 days. Newer KDIGO criteria improved the recognition of AKI, raising the incidence to 58.6% and shortening the median time to detection to 4 days. The KDIGO criteria also allowed for staging of AKI severity. Of the 95 cases of AKI detected by the KDIGO criteria, 52 (54.7%) were stage 1, 28 (29.5%) were stage 2, and 15 (15.8%) were stage 3; 11 of the 15 stage 3 cases were dialyzed. AKI occurred more frequently, earlier and reached higher stages in patients using the deoxycholate versus the liposomal preparation. To our knowledge, this is the first study to evaluate the performance of the KDIGO criteria in AmB-induced AKI.

Since the incidence and impact of AKI on outcomes is dependent upon the criteria used to define it (25), we sought to compare our findings with that of prior studies of AmB nephrotoxicity. In those studies, the most commonly used criterion to define renal toxicity was a doubling (or greater) of baseline SCr, which we termed NT2x. This definition is analogous to a KDIGO stage 2 or greater. Applying the NT2x definition to our data, the incidence of AKI secondary to deoxycholate AmB was 31.7%, which is comparable to that found by Nucci (18), Walsh (14) and Johnson (20) (31.8%, 33.7% and 37.5%, respectively); Moreau (26) and Caillot (12) found higher (56.3 and 66.7%, respectively) and Prentice (19) encountered lower (23%) incidences. For the liposomal preparation, we detected AKI by NT2x criteria in 16.7% of patients, which is in agreement with the findings of Walsh (14) (18.7%) and slightly

higher than the incidences detected by Leenders (27), Prentice (19) and Johnson (20)(11.8%, 11.1% and 9.4%, respectively).

Using the KDIGO criteria, however, we detected AmB-induced AKI in 60.0% of patients using deoxycholate and 54.8% of patients using liposomal. These incidences are significantly higher than those previously described, especially for the liposomal preparation. In fact, several AKI episodes must have gone clinically undetected because no changes in management in response to AKI were made in approximately 1/3 of patients (36/95, 37.9%) and a Nephrology consult was requested in less than 1/3 of patients with AKI (25/95, 26.3%).

Minejima et al. showed that the AKIN criteria were more sensitive than traditional criteria in detecting vancomycin nephrotoxicity (21). In their opinion, early detection of vancomycin nephrotoxicity has the potential to improve management because it may lead to changes in management that halt the process of renal injury. A similar rationale could be applied to AmB-induced AKI. Nevertheless, since our data are observational, prospective studies would be needed to confirm this hypothesis. A potential drawback of using a highly sensitive AKI criterion would be to discontinue or reduce AmB dose unnecessarily, potentially interfering with the management of the underlying infection. In the present study, KDIGO stage 1 AKI was not predictive of need for ICU admission, need for mechanical ventilation or mortality. In fact, these outcomes were numerically less common in patients with stage 1 AKI than in those without AKI. When AKI was defined by traditional criteria, however, there was a significant association with all of these outcomes.

Independent predictors of AKI also varied according to the definition used. AKI by KDIGObin was predicted by older age and use of furosemide (logistic regression) and ACE-I (Cox regression). AKI by NT0.5 was predicted by use of older age and use of vancomycin, whereas the more severe episodes of AKI defined by NT2x criteria were predicted by use of furosemide and vasopressors. Chertow and coworkers (28) identified ICU admission and use of cyclosporine as independent predictors of AKI. In our study, ICU admission predicted AKI by traditional criteria on univariate analysis, but was removed from the model after adjustment for other confounders. We did not have patients on cyclosporine for comparison.

Our study has several limitations. We applied newer diagnostic criteria for AKI to retrospective data. Our ability to detect AKI may have been negatively influenced by the fact that we did not have daily serum creatinine values for all patients and did not incorporate urine output data. Indeed, the AKIN criteria could not be applied to 25 patients due to missing creatinine values at critical time points. Although we did not have formal data on fluid responsivity or renal imaging for all patients, we made an effort to exclude pre or post renal causes of AKI by thoroughly reviewing EMRs and paper charts. Finally, since some patients were exposed to other renal insults, ischemic and/or toxic, we cannot state that use of AmB was the sole cause of AKI in all subjects.

In summary, we demonstrated that the newer AKI criteria are more sensitive than traditional criteria to detect AmB-induced AKI. As expected, this improved sensitivity occurred at the expense of potential overdiagnosis of mild cases. Whether or not this increased sensitivity of newer AKI criteria will

translate into earlier interventions and better renal and patient outcomes remains unproven. In our dataset, mild cases of AKI were not predictive of worse outcomes. Potential drawbacks of overly sensitive AKI criteria also need to be considered because undue AmB discontinuation or dose reduction could compromise the treatment of the underlying disease.

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CHAPTER / MANUSCRIPT 2

TITLE PAGE

Title: Role of Urine Neutrophil Gelatinase-Associated Lipocalin (NGAL) in the Early Diagnosis of Amphotericin B-induced Acute Kidney Injury

Running title: Urine NGAL in Amphotericin B-induced AKI

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ABSTRACT

Neutrophil Gelatinase-Associated Lipocalin (NGAL) detects acute kidney injury (AKI) earlier than serum creatinine (SCr) in settings such as cardiac surgery, contrast nephropathy and intensive care units. We hypothesized that urine NGAL (UrNGAL) would be an early biomarker of drug nephrotoxicity. To test this, we studied hemodynamically stable patients treated with Amphotericin B (AmB). We measured SCr and UrNGAL at baseline and daily after initiation of AmB up to day 14 or development of AKI by SCr criteria. AKI was defined according to Kidney Diseases Improving Global Outcomes (KDIGO) criteria (increase in SCr by ≥0.3 mg/dl within 48 hours or ≥1.5 times baseline within 7 days). We studied 24 patients with a mean age of 48.4 ± 16.4 years. Most were male and received AmB (12 deoxycholate and 12 liposomal) for the treatment of leishmaniasis (91.7%). Overall, 17/24 patients fullfilled KDIGO criteria for AKI. Peak UrNGAL levels were higher in AKI than in No AKI patients and in recipients of deoxycholate than liposomal AmB. The diagnostic performance of UrNGAL on day 5 to detect AKI was moderate, with an AUC 0.68 (95% CI 0.41 to 0.95). In the deoxycholate subgroup, however, the AUC rose to 0.89 (95% CI 0.67 to 1.00). In a patient-level analysis, we found that UrNGAL was able to detect AKI 3.2 days earlier than SCr (3.7 ± 2.5 versus 6.9 ± 3.3 days, time to AKI by UrNGAL and SCr criteria, respectively; p = 0.001). Future studies should evaluate if a UrNGAL-oriented treatment strategy will improve outcomes.

INTRODUCTION

Acute kidney injury (AKI) is a frequent clinical syndrome in hospitalized patients (1). The incidence of AKI varies widely across studies, mostly due to heterogeneity of the clinical settings and of the criteria used to define the syndrome. Nevertheless, the global incidence of AKI appears to be increasing (2). There are many recognized etiologies of AKI but most cases are due to ischemic and / or toxic insults (3). AKI is associated with a number of adverse short-term outcomes, including mortality (4). More recently, episodes of AKI have been linked to development of chronic kidney disease and progression of chronic kidney disease to end-stage renal disease (5).

Several interventions that attenuate AKI in experimental models have been proven ineffective when carried into clinical practice (6). In part, this inefficiency has been attributed to late detection of AKI, after the occurrence of irreversible acute tubular necrosis. In 2002, a panel of experts entitled Acute Dialysis Quality Initiative began efforts to institute a uniform definition of AKI that would detect the syndrome at an earlier stage (7). This group initially published the RIFLE (Risk, Injury Failure, Loss, End-stage) criteria (8) that were later modified by experts from the Acute Kidney Injury Network (AKIN) into the so-called AKIN criteria (9). More recently, RIFLE and AKIN were consolidated into the Kidney Diseases Improving Global Outcomes (KDIGO) criteria (10). According to KDIGO, AKI is defined as an increase in serum creatinine (SCr) by ≥ 0.3 mg/dl within 48 hours; or an increase in SCr to ≥ 1.5x baseline, which is known or presumed to have occurred within the

prior 7 days; or urine volume < 0.5 ml/kg/hour for 6 hours (11). In a retrospective cohort study of 2579 critically ill patients, KDIGO outperformed AKIN criteria, showing improved sensitivity to detect AKI without compromising specificity (12).

Nevertheless, as it relies on SCr and urine volume, the KDIGO criteria might still have several limitations. Adequate measurement of urine volume, for example, may not occur without a bladder catheter, especially outside the intensive care setting. Additionally, a significant proportion of AKIs are non-oliguric. Finally, the use of loop diuretics may invalidate urine volume as a marker of AKI. SCr also has significant restrictions because its levels can be affected by factors unrelated to the glomerular filtration rate, such as catabolism, rhabdomyolysis, certain antibiotics, hemodilution and muscle mass. In addition, a rise in SCr requires a significant decrease in glomerular filtration rate and is thus considered a late marker for AKI. In the last decade, there has been an intense search for early biomarkers of AKI (13). In humans, the AKI biomarker that has been most studied is neutrophil gelatinase–associated lipocalin (NGAL) (14).

NGAL is a member of the lipocalin superfamily. These proteins are composed of 8 β-strands that form a β-barrel encircling a calyx. In 2003, Mishra et al. demonstrated that NGAL protein is markedly overexpressed in the proximal tubules of early ischemic mouse kidneys. More importantly, NGAL protein could be easily detected in the urine immediately after mild renal ischemia in mice; this was also reproduced in rats and in a model of nephrotoxic (cisplatin) kidney injury (15). In 2005, the same group of investigators were

able to show that in children submitted to cardiopulmonary bypass, urine or serum NGAL levels two hours after surgery were able to predict those patients who would go on to develop AKI by SCr criteria (16). Although the role of NGAL overexpression following tubular injury is not completely elucidated, there is some evidence that it might be protective (17) by aiding the cell proliferation-repair process (18).

Most NGAL studies in humans have been performed in the context of large surgeries (16, 19, 20), contrast nephropathy (21–25) and in critically ill patients (26–29); collectively, they suggest that NGAL is a promising early biomarker of AKI. However, there are no intervention studies demonstrating that the early detection of renal damage in these situations results in reduced incidence of clinically manifest AKI or in associated morbidity and mortality (30). In fact, early detection of kidney injury after a single, isolated insult, such as after heart surgery or a single dose of iodinated contrast, may have little practical value, because the full insult has already occurred. In sepsis or septic shock, although insults are ongoing, early recognition of AKI may not translate into better management if the physician is already doing everything possible to reverse the hemodynamic abnormalities that are causing kidney damage. Furthermore, the inflammatory environment of sepsis may interfere with the diagnostic performance of NGAL, as it may increase regardless of renal injury (31–34).

One promising application for biomarkers of AKI, however, is monitoring drug nephrotoxicity (35–37). Once a nephrotoxic insult causes irreversible acute tubular necrosis, it usually takes 7 to 21 days for renal function to recover,

even after the offending agent is stopped. In this setting, early knowledge of ongoing AKI may enable the physician to take measures to avoid additional damage before the progression to full blown acute tubular necrosis. Preliminary studies in rodents have shown that NGAL is a promising biomarker of AKI secondary to cisplatin (15, 38), amphotericin B (AmB) (39), colistin (40) and gentamicin (41), but few studies have explored this application in humans.

In 12 patients with cancer receiving cisplatin infusion, urinary NGAL (UrNGAL) rose 4.5 days earlier than the peak SCr (42). However, in patients with acute bacterial infections, the ability of plasma NGAL and UrNGAL to predict the nephrotoxicity of vancomycin (43) and colistin (44), respectively, was compromised.

Aiming to evaluate the role of UrNGAL as an early biomarker of drug-induced AKI, we used the treatment of non-septic, hemodynamically stable patients with AmB as a unique model. At our institution, leishmaniasis is the main indication for AmB on the hospital wards. These patients usually do not present with an inflammatory response syndrome or renal dysfunction, are hemodynamically stable (and hence not subject to ischemic insults) and require in-house treatment with AmB for prolonged periods. AmB is a highly nephrotoxic drug with antiparasitic and antifungal properties. A recent meta-analysis indicated that 32.5% of patients treated with the deoxycholate formulation and 14.5% treated with the liposomal formulation develop AKI (45). However, these average incidences of AKI reported in this meta-analysis came from studies conducted more than 10 years ago, involving

heterogeneous populations and using various definitions of AKI. More importantly, none of them used currently accepted AKI criteria. Unpublished data from a retrospective cohort study by our group, that included 162 (120 deoxycholate and 42 liposomal) inpatients treated with AmB, revealed an overall incidence of AKI of 58.6% when using the KDIGO criteria. Moreover, KDIGO criteria detected AmB-induced AKI earlier than traditional criteria. Kondo et al. have shown that the genes for NGAL are upregulated after treatment with AmB (39) but to date, there are no human studies evaluating the role of NGAL as an early biomarker of AmB nephrotoxicity.

MATERIALS AND METHODS

Site: Hospital Professor Edgard Santos, a tertiary care facility affiliated with the Medical School of the Federal University of Bahia, located in Salvador, Bahia, Brazil.

Population: All adult inpatients initiating treatment with AmB in the medical wards were considered potentially eligible. We did not include intensive care unit patients, as they are usually exposed to ischemic renal insults due to septic or cardiogenic shock. Participants with urinary tract infection, status post-renal transplantation, with ongoing acute kidney injury, advanced (stage IV or stage V) chronic kidney disease, and who used AmB for less than 3 days were excluded.

Design: prospective cohort study of adult inpatients treated with AmB. All patients were followed from initiation of AmB until death or hospital discharge. From the viewpoint of treatment, the study was observational,

without any deviation from standard practice. The indication for treatment as well as the dose and type of AmB were the responsibility of the medical staff assisting the patient, without any interference from the research team. Similarly, decisions to discontinue or reduce the dose of AmB were prerogatives of the primary care team. Our intervention was purely diagnostic. Blood and urine samples were collected prior to initiation of AmB therapy and daily thereafter until day 14 or development of AKI by SCr criteria using the KDIGO definition. The primary care team had no knowledge of UrNGAL levels because testing was done in batches at a later point in time.

Protection of human subjects: Our hospital's institutional review board approved the study (protocol number 08087412.1.0000.0049) and all participants provided written informed consent.

Measurements: We collected data on demographics, date of admission, indication for AmB use, type and dose of AmB, comorbid conditions, use of other potentially nephrotoxic drugs, and daily blood and urine samples for laboratory data.

Laboratory methods: Blood samples were collected in the morning by the hospital's laboratory personnel through peripheral venipuncture and sent immediately to our hospital's clinical laboratory for analysis of SCr, potassium, magnesium and bicarbonate, according to the usual routine for inpatients. Twenty ml of freshly voided urine was collected in the morning by the research team; ten ml was sent to the hospital clinical laboratory for urine creatinine and the remaining ten ml was immediately stored in a -80^{o}C freezer (SANYO Vip Series). Frozen urine samples were shipped on dry ice to a

commercial laboratory (Science Pro laboratories, São Caetano do Sul, Brazil). Urine samples were centrifuged and supernatants diluted 1/50 for UrNGAL measurement using NGAL Rapid ELISA Kit (catalog number KIT037RUO) according to manufacturer's instructions (BioPorto Diagnostics, Gentofte, Denmark). Each subject's longitudinal samples were assayed in the same batch. Quantitative UrNGAL results were obtained in ng/ml (assay range 0.2-20.0 ng/ml, limit of detection < 0.1 ng/ml).

AmB treatment regimen: At our hospital, the usual protocol for initiating deoxycholate AmB is to start with 0.25 mg/kg/d and increase 5-10 mg/day to a maximum of 1.5 mg/kg/d. For the liposomal preparation, we start with 3-5 mg/kg/d. Both are diluted in 500 ml of 5% dextrose in water and infused intravenously over 1-2 hours. In addition, patients receive 500 to 2000 ml of 0.9% NaCl over 24 hours, at the discretion of the attending physician. Pretreatment with acetaminophen, anti-histamines or hydrocortisone is reserved for patients who develop reactions during infusion.

Definitions: AKI was defined according to the KDIGO criteria, which requires an absolute increase in SCr by ≥ 0.3 mg/dl within 48 hours; or an increase in SCr to ≥ 1.5x baseline, which is known or presumed to have occurred within the prior 7 days (binary definition, hereafter abbreviated as KDIGObin, equivalent to KDIGO stage 1 or greater). We did not have rigorous measurement of urine volume. AKI was staged according to KDIGO: stage 1 when there was absolute increase in SCr ≥ 0.3 mg/dl or an increase in SCr 1.5-1.9x baseline; stage 2 required an increase in SCr 2.0-2.9x baseline and stage 3 an increase in SCr to ≥ 3.0x baseline or initiation of dialysis. For comparison,

we examined two definitions of AmB nephrotoxicity commonly used in the literature: an absolute increase in SCr by ≥ 0.5 mg/dl (NT0.5) and doubling of baseline SCr (NT2x), the latter being equivalent to a KDIGO stage 2 or greater. There is currently no consensus definition of AKI based on UrNGAL levels.

Outcomes: The primary endpoint was the difference between the average time to detection of AKI by SCr criteria (KDIGO) versus empirically-derived UrNGAL criteria. We also evaluated the sensitivity, specificity, positive and negative predictive value and accuracy of UrNGAL for the detection of AmB-induced AKI.

Statistical analyses: Data were summarized by counts, relative frequencies and measures of central tendency and dispersion (mean ± standard deviation or median and interquartile range, according to the shape of the distribution). NGAL data were analyzed in ng/ml and μg/g of urine creatinine. Given the asymmetry of UrNGAL data, we also analyzed log-transformed data. To account for differences in baseline values, we also expressed UrNGAL as a relative value, by dividing values from days 1 thru 14 by the baseline (day 0) value. Correlation between datapoints was assessed with the Spearman's correlation coeficient. Comparisons of continuous variables between two groups were made with the Wilcoxon rank-sum test or, for log-transformed UrNGAL data, the Student's t test. Trend across ordered groups was tested using a nonparametric test developed by Cuzick, which is an extension of the Wilcoxon rank-sum test. Receiver operating characteristics (ROC) curves were generated at all time-points to determine the best sensitivity/specificity cutoffs for the detection of AKI by UrNGAL. Individual patient-level analyses

were also carefully conducted to identify the best UrNGAL cutoff for the early diagnosis of AKI. Two by two tables were constructed and sensitivity, specificity, positive and negative predictive values and accuracy were determined. Time to peak UrNGAL versus time to peak SCr in patients with AKI and time to AKI by empirically-derived UrNGAL criteria versus time to AKI by SCr (KDIGO) criteria in matched pairs of concordant cases (true positives) were compared with the one-sample paired t test. A p value < 0.05 in final analyses was considered statistically significant. All analyses were performed using the Stata 12.1 and IBM SPSS Statistics 20.0 softwares.

Sample size: The minimum sample size required to detect a difference between mean time to AKI by UrNGAL criteria and mean time to AKI by SCr (KDIGO) criteria was calculated using Open Epi, available at http://www.openepi.com/v37/SampleSize/SSMean.htmTh. A priori sample size calculation was based on the following assumptions: mean time to AKI by UrNGAL criteria of 3 ± 1 days and mean time to AKI by SCr criteria of 5 ± 2 days. A sample size of 20 AKI cases would provide us 80% power to detect an average difference of 2 days with an alpha level of 0.05.

RESULTS

Study population

We studied 24 patients, with a mean age of 48.4 ± 16.4 years. Most were male, from rural areas and received AmB (12 deoxycholate and 12 liposomal) predominantly for the treatment of leishmaniasis (91.7%). Three patients had HIV. Mean length of stay in the hospital was slightly over one month. Mean

baseline renal function, acid-base status and electrolytes were within normal limits (Table 2.1).

At baseline, UrNGAL levels were low in the majority of patients with a median [IQR] of 0.23 [0.11 to 0.93] ng/ml; in 6 patients, baseline UrNGAL was above 1.0 ng/ml; one of them had a baseline level of 13.9 ng/ml in the absence of clinically manifest AKI. Due to the skewness of UrNGAL data, we also performed log transformation (Table 2.1).

Summary of AmB treatment

For deoxycholate AmB, the mean starting, maintenance and total doses were 26 ± 10 mg (min. 15 mg/d; max. 50 mg/d), 47 ± 18 mg (min. 15 mg/d; max. 75 mg/d), and 730 ± 731 mg, respectively (min. 75 mg/d; max. 2485 mg/d). For liposomal AmB, the mean starting, maintenance and total doses were 116 ± 38 mg (min. 50 mg/d; max. 150 mg/d), 211 ± 85 mg (min. 50 mg/d; max. 300 mg/d) and 2560 ± 1314 mg, respectively (min. 450 mg/d; max. 5250 mg/d).

Incidence and time to AKI by SCr criteria

We sought to establish the most sensitive SCr-based criteria for AKI. First, we examined two traditional definitions of nephrotoxicity. By NT2x criterion, 4/24 (16.7%) patients developed AKI (3/12 deoxycholate and 1/12 liposomal) 10.3 ± 3.4 days after initiating AmB. Peak SCr in those who developed AKI by NT2x criterion was 1.8 ± 0.4 mg/dl, occurring 10.8 ± 2.6 days after initiation of AmB.

Table 2.1. Demographic, clinical and baseline laboratory variables in 24 patients treated with AmB.

Variables	All (n = 24)
Age (years), mean±SD	48.4 ± 16.4
Gender	
Male	19/24 (79.0%)
Female	5/24 (21.0%)
Place of residence	
Salvador (capital)	3/24 (12.5%)
Rural areas	21/24 (87.5%)
Reason for AmB treatment	
Leishmaniasis*	22/24 (91.7%)
Histoplasmosis	1/24 (4.17%)
Paracoccidioidomycosis	1/24 (4.17%)
Significant comorbidities	
HIV	3/24 (12.5%)
Systemic lupus erythematosus	1/24 (4.17%)
Hemophagocytic syndrome	1/24 (4.17%)
Cirrhosis	1/24 (4.17%)
AmB formulation	
Deoxycholate	12/24 (50.0%)
Liposomal	12/24 (50.0%)
Length of stay in the hospital (days), mean±SD	36.2 ± 15.4
Baseline serum laboratory values	
Serum creatinine (mg/dl)	0.8 ± 0.2
Serum potassium (meq/l)	4.3 ± 0.6
Serum magnesium (mg/dl)	2.0 ± 0.3
Serum bicarbonate (meq/l	28.0 ± 4.8
Baseline urine laboratory values	
Urine creatinine (mg/dl), n = 24	102.2 ± 69.2
Baseline UrNGAL strata	
0.01 |– 1.00 ng/ml	18/24 (75.0%)
1.00 |– 2.00 ng/ml	3/24 (12.5%)
≥ 2.00 ng/ml	3/24 (12.5%)
Baseline UrNGAL	
UrNGAL (ng/ml)	1.2 ± 2.9
Log UrNGAL (ng/ml)	-1.2 ± 1.7
UrNGAL (µg/g creatinine)	3.1 ± 11.0
Log UrNGAL (µg/g creatinine)	-1.1 ± 1.9

Legend: AmB = amphotericin B; UrNGAL = urine neutrophil gelatinase-associated lipocalin. *Of the 22 cases of leishmaniasis, 5 were visceral (kalazar) and 17 were tegumentary (localized cutaneous leishmaniasis = 2; disseminated cutaneous leishmaniasis = 6; mucosal leishmaniasis = 3; disseminated cutaneous leishmaniasis with mucosal involvement = 6)

Using NT0.5 criterion, we were able to diagnose 11 additional cases, raising the incidence of AKI to 62.5% (15/24), 9/12 deoxycholate and 6/12 liposomal, and reducing time to detection to 8.0 ± 3.4 days. Peak SCr in those who developed AKI by NT0.5 was 1.7 ± 0.4 mg/dl, occurring 8.3 ± 3.5 days after initiation of AmB.

Table 2.2. Incidence of AKI according to different definitions, stratified by type of AmB.

AKI criteria	All (n = 24)	Type of AmB	
		Deoxycholate (n = 12)	Liposomal (n = 12)
Binary			
NT2x	4/24 (16.7%)	3/12 (25.0%)	1/12 (8.3%)
NT0.5	15/24 (62.5%)	9/12 (75.0%)	6/12 (50.0%)
KDIGO	17/24 (70.8%)	10/12 (83.3%)	7/12 (58.3%)
KDIGO stages			
Stage 1	13/24 (54.2%)	8/12 (66.7%)	6/12 (50.0%)
Stage 2	4/24 (16.7%)	3/12 (25.0%)	1/12 (8.3%)
Stage 3	0/24 (0.0%)	0/12 (0.0%)	0/12 (0.0%)

Legend: AmB = Amphotericin B. NT2x = traditional nephrotoxicity criterion that requires at least doubling of the SCr. NT0.5 = traditional nephrotoxicity criterion that requires an absolute increase in SCr of at least 0.5 mg/dl. KDIGO = kidney diseases improving global outcomes criterion that requires an increase in SCr by ≥ 0.3 mg/dl within 48 hours; or an increase in SCr to ≥ 1.5x baseline, which is known or presumed to have occurred within the prior 7 days. A KDIGO stage 1 required an increase in SCr by ≥ 0.3 mg/dl or 1.5-1.9x baseline; stage 2 required an increase in SCr 2.0-2.9x baseline and stage 3 an increase in SCr to ≥ 3.0x baseline or initiation of dialysis.

Finally, using the KDIGObin criterion, we detected 17/24 (70.8%) cases of AKI, 10/12 deoxycholate and 7/12 liposomal, reducing time to detection to 7.2 ± 3.1 days. Peak SCr in those who developed AKI by KDIGObin was 1.6 ± 0.4 mg/dl, occurring 8.2 ± 3.4 days after initiation of AmB (Tables 2.2 and 2.3). AKI and peak SCr occurred earlier in the deoxycholate than in the liposomal group, regardless of the criteria used.

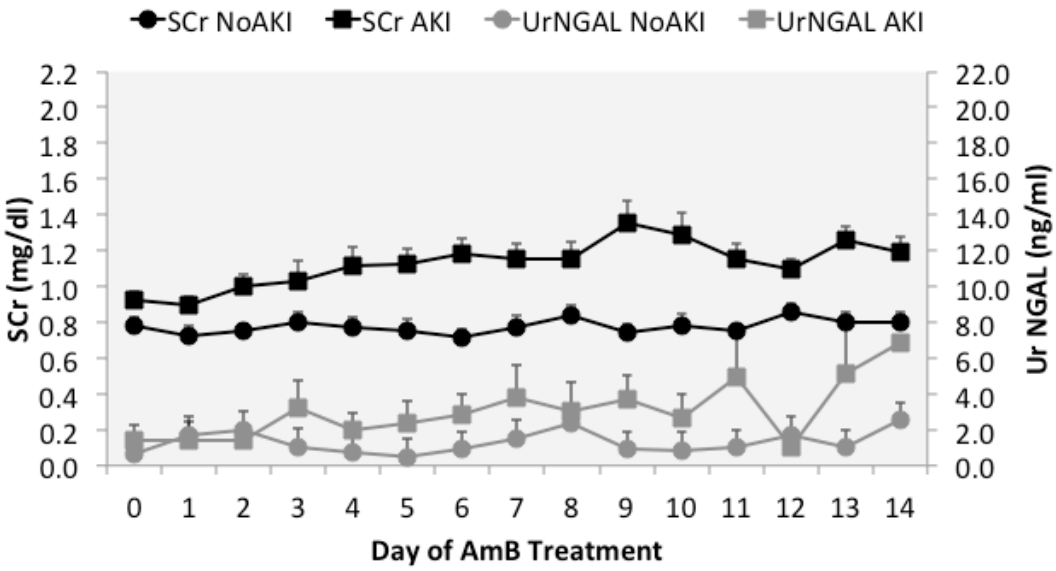
Table 2.3. Time to AKI and time to peak SCr and peak UrNGAL in AKI patients according to different definitions, stratified by type of AmB preparation.

AKI criteria	All	Type of AmB	
		Deoxycholate	Liposomal
Time to AKI, days			
NT2x	10.3 ± 3.4 (n=4)	9.3 ± 3.5 (n=3)	13.0 (n=1)
NT0.5	8.0 ± 3.4 (n=15)	7.3 ± 3.5 (n=9)	9.0 ± 3.4 (n=6)
KDIGO	7.2 ± 3.1 (n=17)	7.1 ± 3.6 (n=10)	7.3 ± 2.6 (n=7)
UrNGAL ≥ 2.54 ng/ml*	5.1 ± 3.6 (n=11)	5.5 ± 3.5 (n=8)	4.0 ± 4.4 (n=3)
UrNGAL ≥ 3x baseline*	3.7 ± 2.5 (n=13)	3.5 ± 2.2 (n=10)	4.3 ± 4.0 (n=3)
Time to peak, days			
SCr, NT2x	10.8 ± 2.6 (n=4)	10.0 ± 2.7 (n=3)	13.0 (n=1)
SCr, NT0.5	8.3 ± 3.5 (n=15)	7.8 ± 3.5 (n=9)	9.2 ± 3.6 (n=6)
SCr, KDIGO	8.2 ± 3.4 (n=17)	8.0 ± 3.4 (n=10)	8.6 ± 3.7 (n=7)
UrNGAL, ng/ml	6.8 ± 3.3 (n=17)	6.3 ± 3.5 (n=10)	7.4 ± 3.2 (n=7)
UrNGAL, % change	6.8 ± 3.3 (n=17)	6.3 ± 3.5 (n=10)	7.4 ± 3.2 (n=7)

Legend: data expressed as mean ± standard deviation. AmB = Amphotericin B. NT2x = traditional nephrotoxicity criterion that requires at least doubling of the SCr. NT0.5 = traditional nephrotoxicity criterion that requires an absolute increase in SCr of at least 0.5 mg/dl. KDIGO = kidney diseases improving global outcomes criterion that requires an increase in SCr by ≥ 0.3 mg/dl within 48 hours; or an increase in SCr to ≥ 1.5x baseline, which is known or presumed to have occurred within the prior 7 days. UrNGAL = urine neutrophil gelatinase-associated lipocalin. * Time to AKI by UrNGAL was analyzed in patients that fulfilled KDIGO and UrNGAL criteria for AKI (true positives).

Figure 2.1a shows mean SCr and UrNGAL data over time for the entire group of 24 patients, stratified by the presence or absence of AKI according to KDIGObin. Mean SCr among groups were similar at days 0, 1 and 3; at day 2 and from day 4 until day 14, SCr levels were significantly higher in the AKI group ($p \leq 0.04$ for days 2 and 8; $p \leq 0.01$ for all other time-points). The highest SCr value of our dataset was 2.2 mg/dl, reached on day 9 in a patient receiving deoxycholate AmB. Figure 2.1b shows mean SCr and Ur NGAL data over time for the entire group of 24 patients, stratified by the type of AmB preparation. Mean SCr levels were significantly higher in the deoxycholate than in the liposomal group on days 4, 10, 11 and 12 (p values = 0.02, 0.01, 0.03, and 0.01, respectively).

a) Stratified by AKI according to KDIGO



b) Stratified by type of AmB preparation

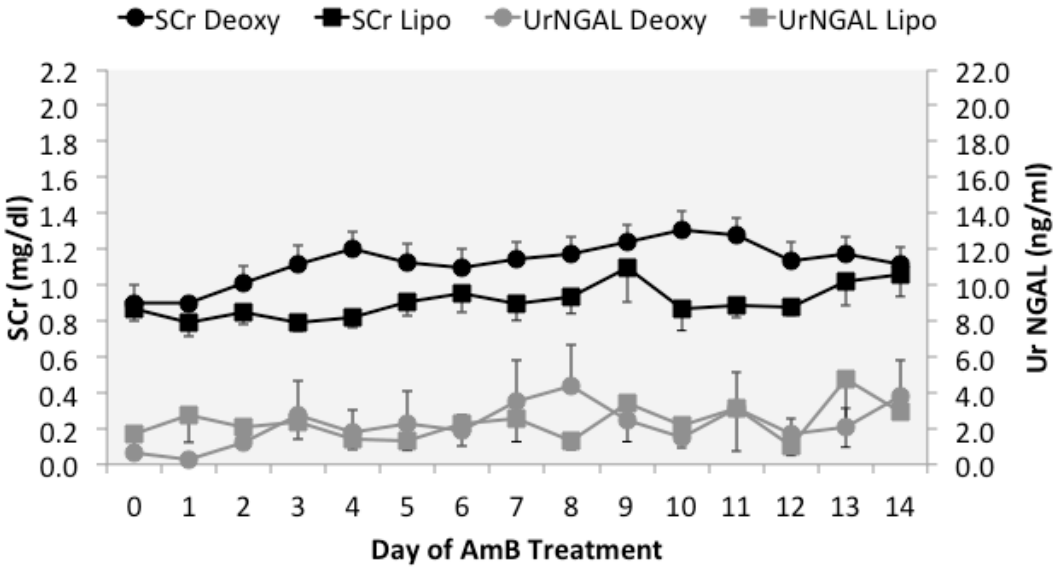


Figure 2.1. Mean SCr and UrNGAL values during two weeks of treatment with intravenous AmB. Legend: SCr = serum creatinine; Ur NGAL = urine neutrophil gelatinase-associated lipocalin; AKI = acute kidney injury; AmB = amphotericin B; Deoxy = deoxycholate; Lipo = liposomal; KDIGO = kidney diseases improving global outcomes criterion that requires an increase in SCr by ≥ 0.3 mg/dl within 48 hours; or an increase in SCr to ≥ 1.5 times baseline, which is known or presumed to have occurred within the prior 7 days. Symbols (squares and circles) represent the mean and bars the standard error of the mean. SCr values (black) are plotted on the primary y axis (left) and Ur NGAL (gray) values on the secondary y axis (right). a) The AKI group is represented by squares and the no AKI group by circles. b) The Lipo group is represented by squares and the Deoxy group by circles.

UrNGAL data

Two-hundred and fifty-six UrNGAL measurements were performed over 14 days in 24 patients. We analyzed UrNGAL data in ng/ml and also normalized by urine creatinine excretion (μg/g urine creatinine) or by baseline values (% change from baseline).

There was a strong correlation between UrNGAL in ng/ml and μg/g creatinine at all time points (Spearman's rho > 0.80 for days 0, 2, 9 and 13; Spearman's rho > 0.90 for all other time points), with consistently higher levels in μg/g than in ng/ml (mean 2.9x higher, range 1.6x to 5.1x on days 9 and 11, respectively). However, standardized dispersion (SD/mean) was also higher at all time points for UrNGAL in μg/g creatinine versus ng/ml (mean 1.4x higher, range 1.1x to 1.7x on days 5 and 4, respectively). Since the diagnostic performance of UrNGAL was not improved by normalizing by urine creatinine, we chose to report the results in ng/ml.

The correlation between UrNGAL in ng/ml and % increase over baseline was weaker (Spearman's rho coefficients ranging from 0.27 to 0.60, at days 5 and 3, respectively). Therefore, some analyses are also reported as % change from baseline.

Mean UrNGAL levels (ng/ml) were not significantly different at any point in time when stratified by the presence or absence of AKI by KDIGObin criterion (Figure 2.1a) or by type of AmB preparation (Figure 2.1b) ($p>0.05$ for all comparisons using non-parametric tests). The only comparison that approached statistical significance was that of log-transformed UrNGAL data

on day 5 across AKI groups, when using NT0.5 criterion (-0.12 ± 1.32 versus -1.43 ± 1.62 log ng/ml; AKI versus No AKI groups; p = 0.053).

As shown in Figure 2.2, peak UrNGAL levels were numerically higher in the AKI than in the no AKI group, but the differences in means were not statistically significant (6.70 ± 7.22 versus 2.90 ± 1.90 ng/ml; p=0.33). When stratified by AKI status across AmB subgroups, the highest peak UrNGAL values were observed in AKI patients in the deoxycholate (7.76 ± 7.71 ng/ml) and liposomal subgroups (5.18 ± 6.73 ng/ml), followed by No AKI patients the deoxycholate (3.12 ± 2.40 ng/ml) and liposomal subgroups (2.81 ± 1.92 ng/ml) (p for trend across ordered groups 0.15).

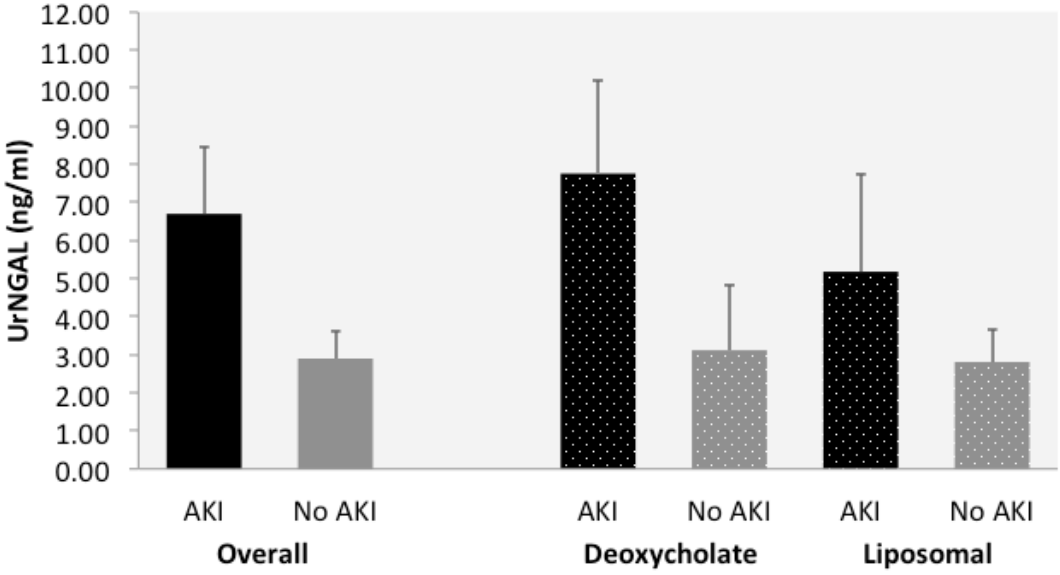


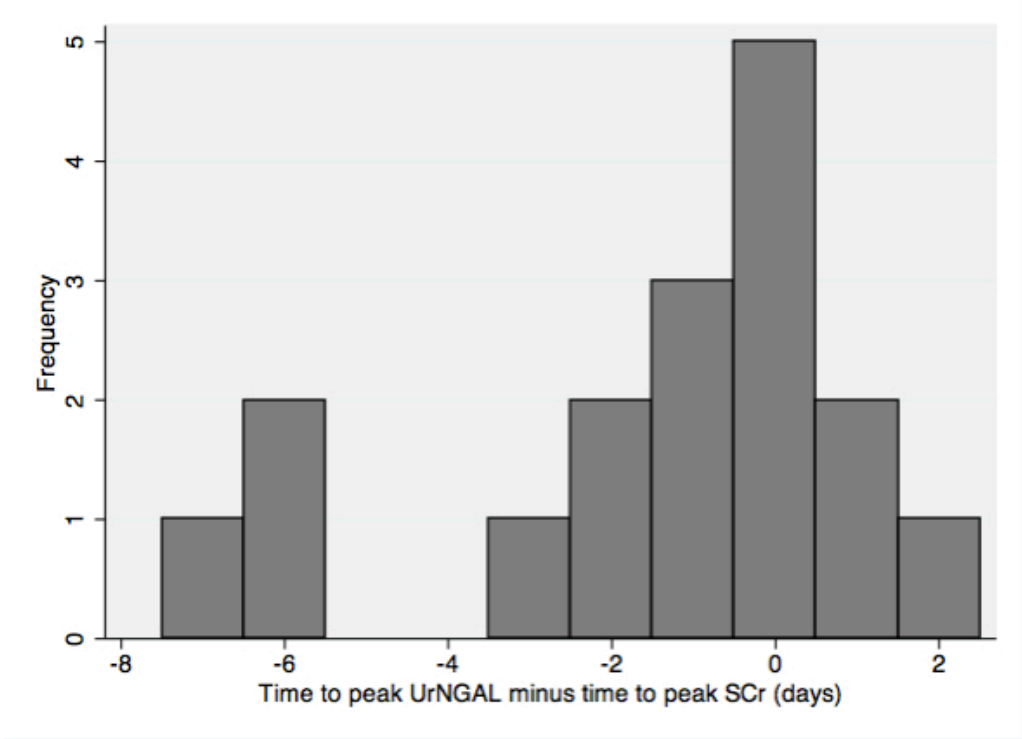
Figure 2.2. Peak UrNGAL levels stratified by AKI status according to KDIGO. Legend: data presented as mean and standard error of the mean. UrNGAL = urine neutrophil gelatinase-associated lipocalin; AKI = acute kidney injury; KDIGO = kidney diseases improving global outcomes criterion that requires an increase in SCr by ≥ 0.3 mg/dl within 48 hours; or an increase in SCr to ≥ 1.5x baseline, which is known or presumed to have occurred within the prior 7 days. Number of cases per group: AKI = 17; No AKI = 7; Deoxycholate subgroup, AKI = 10 and No AKI = 2; Liposomal subgroup, AKI = 7 and No AKI = 5. P for trend = 0.15 (order of groups for trend test: Deoxycholate AKI -> Liposomal AKI -> Deoxycholate no AKI -> Liposomal No AKI).

The highest UrNGAL level in our dataset was 21.88 ng/ml, achieved on day 3 in a patient who developed AKI while using deoxycholate AmB. The highest increase over baseline UrNGAL in our dataset was 9,150.00%, achieved on day 8, also in a patient receiving deoxycholate AmB.

Time to peak UrNGAL versus time to peak SCr in AKI patients

Figure 2.3a shows the distribution of the variable *time to peak UrNGAL minus time to peak SCr* in the AKI group. In 9 patients, UrNGAL peaked before SCr; in 5 patients, the peaks were simultaneous and in 3 patients, UrNGAL peak occurred after SCr. In average, UrNGAL peaked 1.5 days earlier than SCr (6.8 ± 3.3 versus 8.2 ± 3.4 days; time to peak UrNGAL and SCr, respectively; p = 0.035 one sample, paired t test). Results were identical when looking at UrNGAL in % increase over baseline.

a) Time to peak UrNGAL minus time to peak SCr in AKI patients



b) Time to AKI by UrNGAL (≥ 3x baseline) minus time to AKI by SCr criteria

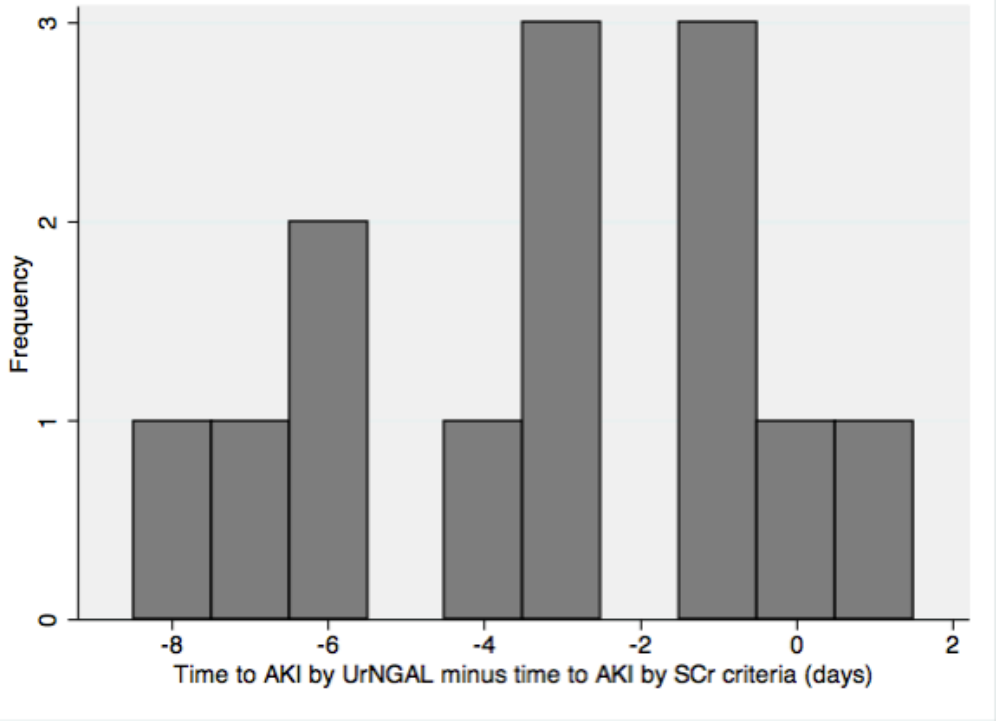


Figure 2.3. Comparison of UrNGAL and SCr in time to peak values and time to AKI. Legend: UrNGAL = urine neutrophil gelatinase-associated lipocalin; SCr = serum creatinine; AKI = acute kidney injury. AKI was defined by KDIGO criterion in all analyses. a) Number of patients = 17. Analyses with time to peak UrNGAL as defined by absolute values (ng/ml) or % change from baseline yielded identical results. b) Number of patients = 13 (concordant AKI diagnosis by both criteria).

Diagnosis of AKI by UrNGAL levels at a specific time-point: ROC curves

To test the diagnostic performance of UrNGAL in detecting AKI using KDIGObin SCr criteria as the gold standard, we performed ROC curves at all time points. UrNGAL levels on day 5 were associated with the highest AUC (0.68; 95% CI 0.41 to 0.95). As shown in Figure 2.4, UrNGAL levels on day 5 were better predictors of AKI when analyzed in absolute (ng/ml) rather than relative values (% change from baseline). However, this performance of UrNGAL on day 5 was more likely due to consistently low UrNGAL values in the No AKI group instead of very elevated levels in the AKI group (0.50 ± 0.49 versus 2.32 ± 5.01 ng/ml; No AKI versus AKI groups, respectively; p = 0.21). A

level of UrNGAL ≥ 0.37 ng/ml on day 5 was associated with a sensitivity of 81%, specificity of 50% and accuracy of 73% in detecting AKI.

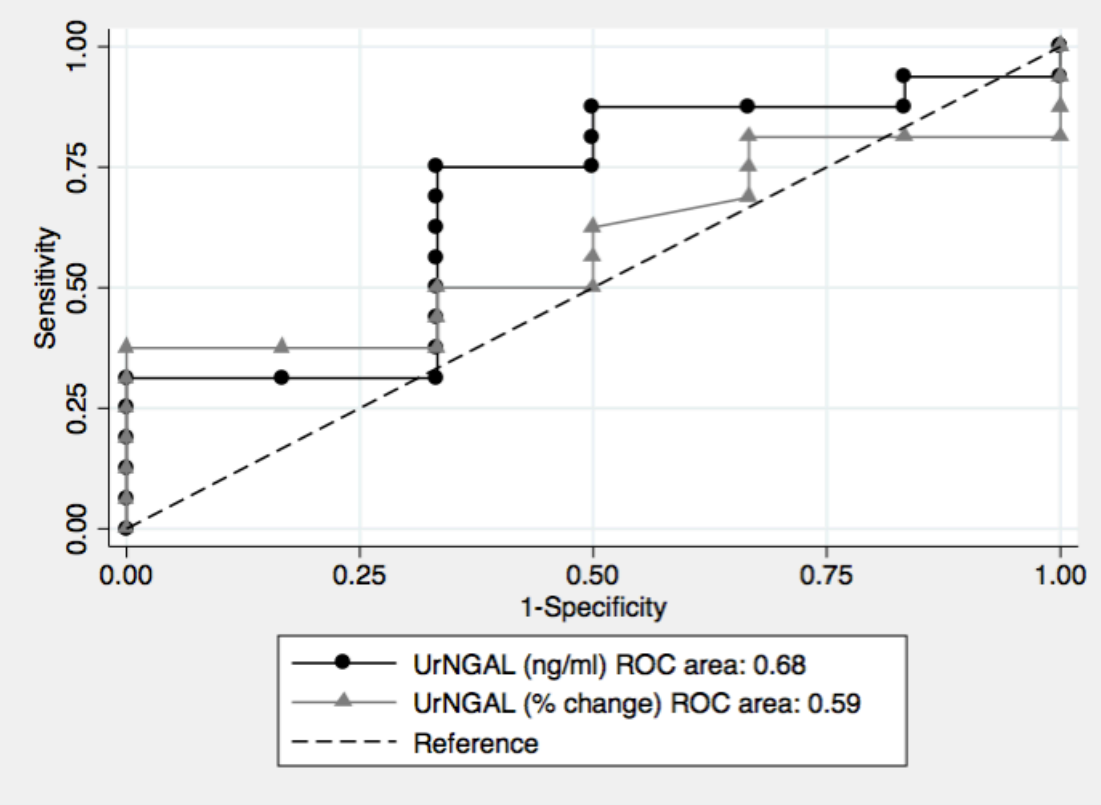


Figure 2.4. Receiver operating characteristics (ROC) curves analyzing the performance UrNGAL on day 5 in detecting AKI using KDIGO SCr criteria as the gold standard. Legend: UrNGAL = urine neutrophil gelatinase-associated lipocalin; SCr = serum creatinine; AKI = acute kidney injury; KDIGO = kidney diseases improving global outcomes criterion that requires an increase in SCr by ≥ 0.3 mg/dl within 48 hours; or an increase in SCr to ≥ 1.5 times baseline, which is known or presumed to have occurred within the prior 7 days. UrNGAL on day 5 was a better predictor of AKI when reported as ng/ml (compared to % change over baseline). All 24 patients (12 deoxycholate and 12 liposomal) are included in this analysis.

We tested whether changing the gold standard used to define AKI cases from KDIGObin to NT0.5 or NT2x would change the diagnostic performance of UrNGAL. For both SCr criteria, UrNGAL levels on day 5 remained the best time-point. However, whereas the use of NT0.5 criterion yielded very similar findings (AUC on day 5 of 0.69; 95% CI 0.42 to 0.95), using NT2x resulted in a

worse diagnostic performance of UrNGAL (AUC on day 5 of 0.46; 95% CI 0.09 to 0.83).

All analyses were repeated using log-transformed UrNGAL data as well as UrNGAL expressed in μg/g of urine creatinine or percent increase over baseline; all of these alternative ways of expressing UrNGAL data resulted in inferior diagnostic performance (data not shown).

Diagnosis of AKI by UrNGAL levels at a specific time-point: subgroup analyses (deoxycholate versus liposomal)

Lastly, we repeated ROC analyses to test the diagnostic performance of UrNGAL (expressed in ng/ml) in the deoxycholate and liposomal subgroups.

In the deoxycholate subgroup, the diagnostic performance of UrNGAL on day 5 was better than when considering the entire group. Using KDIGObin definition as gold standard, the AUC for UrNGAL on day 5 was 0.89 (95% CI 0.67 to 1.00); an UrNGAL level ≥ 0.23 ng/ml was associated with a sensitivity of 88.9%, sensitivity of 100% and accuracy of 90.9%. Using NT0.5 criterion, an UrNGAL on day 5 ≥ 0.23 ng/ml resulted in a perfect fit (AUC = 1.00, 100% sensitivity, specificity and accuracy). This perfect discriminative ability of UrNGAL on day 5 when using NT0.5 as the gold standard was due to consistently low levels in the NoAKI group (UrNGAL 0.07 ± 0.08 versus 3.14 ± 6.88 ng/ml in the NoAKI and AKI groups, respectively; p = 0.01).

In the liposomal group, the diagnostic performance of UrNGAL was worse. Here, the day associated with the best AUC was day 6. The AUC for UrNGAL levels on day 6 was 0.60 (95% CI 0.21 to 0.99); results were the same when using KDIGObin or NT0.5 as gold standard.

Diagnosis of AKI by UrNGAL: patient-level analyses

The UrNGAL cutoffs on day 5 identified by ROC curves were very low; in fact, they were consistent with baseline values. Moreover, by day 5, one quarter of the patients already had the diagnosis of AKI established by SCr KDIGO criteria (time to AKI by KDIGObin = 7.2 ± 3.1 days, median 7 days, IQR 5 to 9 days). In some patients, UrNGAL had already peaked and declined by day 5; in others, UrNGAL levels had not yet risen by day 5. Since we were interested in finding if UrNGAL could detect AKI earlier than SCr, we reviewed individual patient-level data. For these analyses, after carefully observing UrNGAL data, we chose an UrNGAL cutoff of ≥ 2.54 ng/ml as the criterion for AKI.

As illustrated in Table 2.4a, using this cutoff and SCr KDIGObin as the gold standard, we found 11 true positives, 3 false positives, 6 false negatives and 4 true negatives (sensitivity 64.7%; specificity 57.1%; positive predictive value 78.6%; negative predictive value 40.0%; accuracy 62.5%).

Table 2.4. Diagnostic performance of UrNGAL: patient-level analysis

a) Absolute (ng/ml) UrNGAL values

		AKI by SCr (KDIGO criteria)		Total
		Yes	No	
AKI by UrNGAL (≥ 2.54 ng/ml)	Yes	11	3	14
	No	6	4	10
	Total	17	7	24

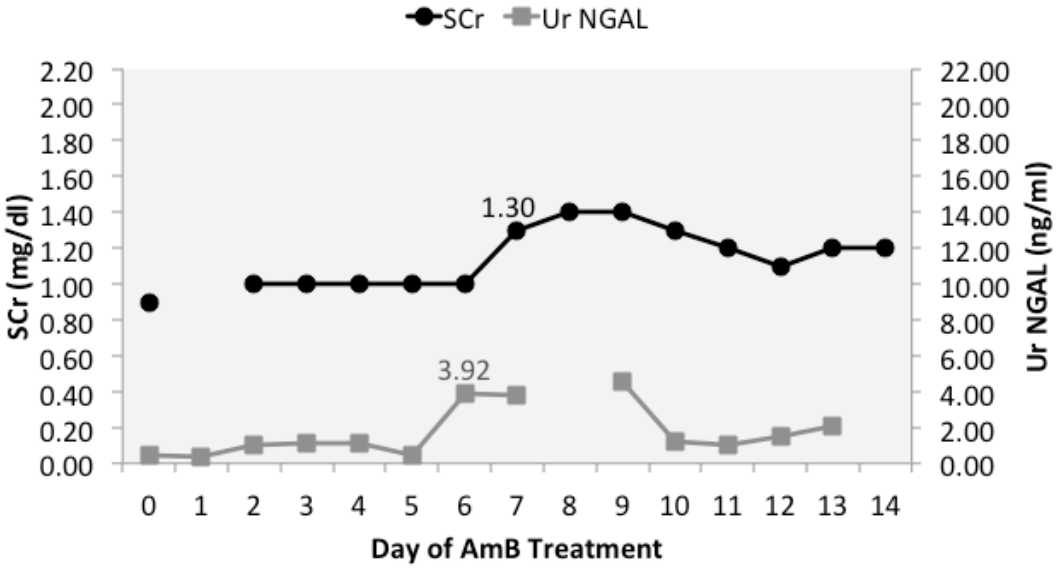
b) Relative (% change from baseline) UrNGAL values

		AKI by SCr (KDIGO criteria)		Total
		Yes	No	
AKI by UrNGAL (≥ 3x baseline)	Yes	13	5	18
	No	4	2	6
	Total	17	7	24

Legend: AKI = acute kidney injury; UrNGAL = urine neutrophil gelatinase-associated lipocalin; SCr = serum creatinine; KDIGO = kidney diseases improving global outcomes criterion that requires an increase in SCr by ≥ 0.3 mg/dl within 48 hours; or an increase in SCr to ≥ 1.5 times baseline, which is known or presumed to have occurred within the prior 7 days.

Figure 2.5 displays two examples of true positive cases.

a) AKI diagnosed 1 day earlier by UrNGAL criteria



b) AKI diagnosed 1 day later by UrNGAL criteria

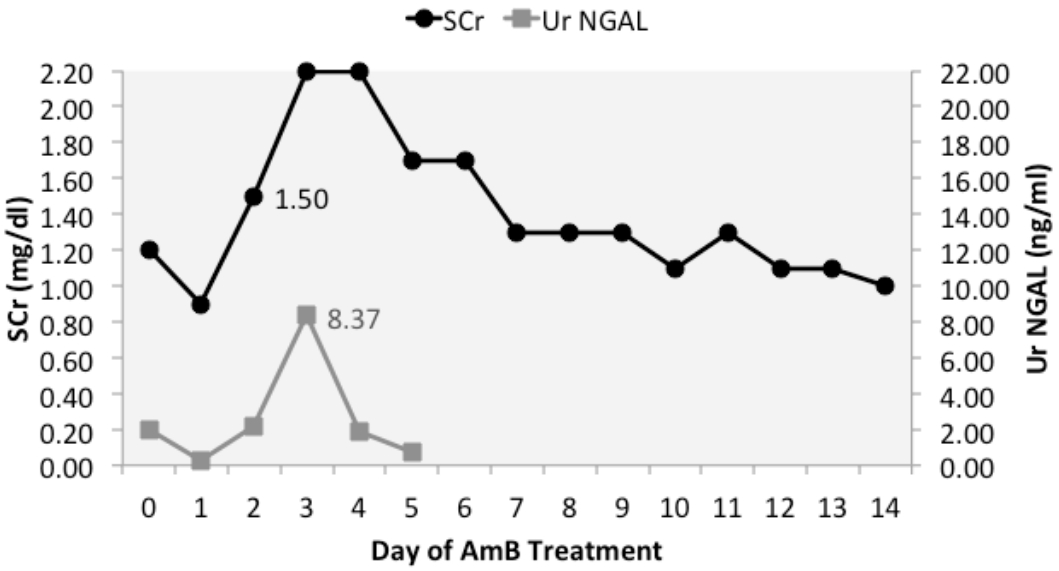
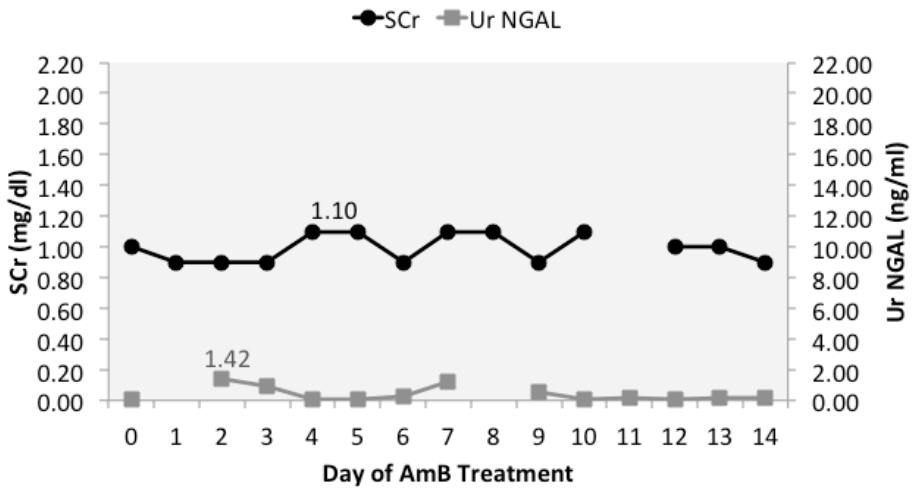
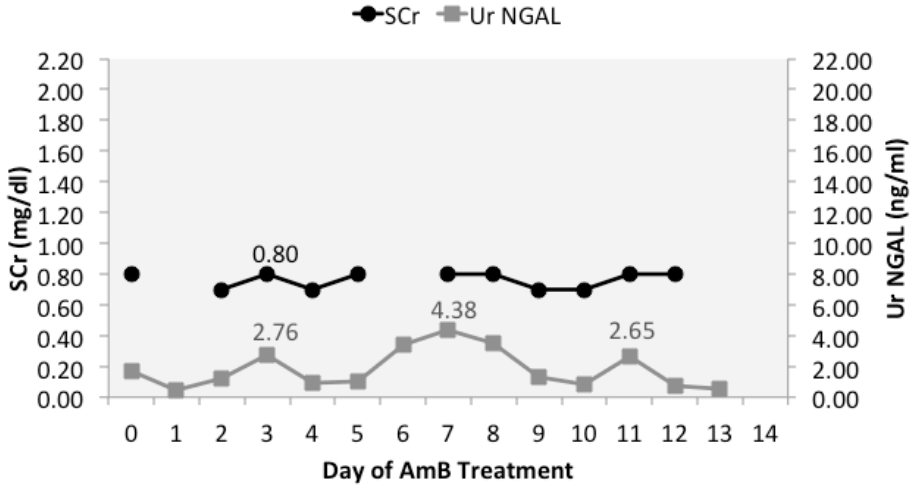


Figure 2.5. Representative examples of true positive cases by UrNGAL (≥ 2.54 ng/ml) criteria, considering SCr (KDIGO) criteria as the gold standard. Legend: AKI = acute kidney injury; UrNGAL = urine neutrophil gelatinase-associated lipocalin; SCr = serum creatinine; KDIGO = kidney diseases improving global outcomes; Amb = amphotericin B.

a) True Negative



b) False Positive



c) False Negative

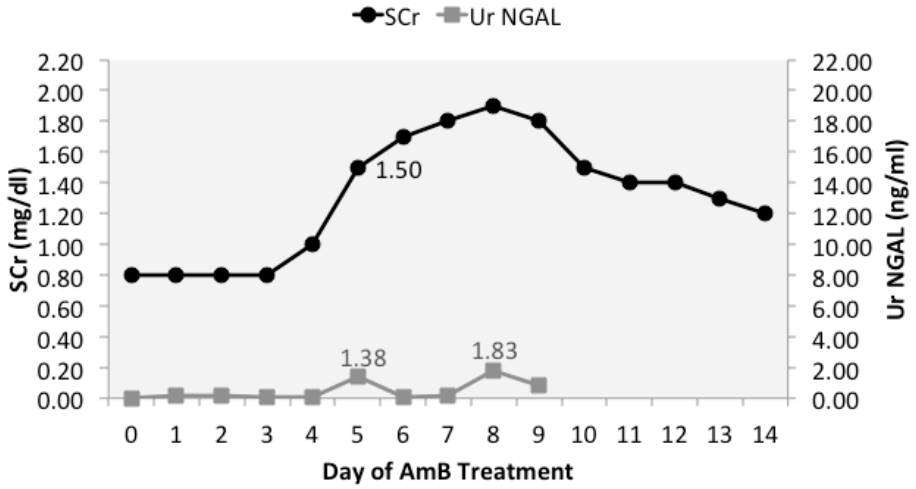


Figure 2.6. Representative examples of true negative, false positive and false negative cases by UrNGAL (≥ 2.54 ng/ml) criteria, with SCr (KDIGO) criteria as the gold standard.
Legend: AKI = acute kidney injury; Ur NGAL = urine neutrophil gelatinase-associated lipocalin; SCr = serum creatinine; KDIGO = kidney diseases improving global outcomes; Amb = amphotericin B.

Figure 2.6 displays representative true negative, false positive and false negative cases. Changing the gold standard to NT0.5 resulted in a worse diagnostic performance due to an increased frequency of false positive cases (from 3 to 5).

We had two cases in which baseline UrNGAL was ≥ 2.54 ng/ml. In the first of these cases, baseline UrNGAL was 4.20 ng/ml, but levels from days 1 thru 5 were all < 1.0 ng/ml. On day 6, UrNGAL increased to 6.5 ng/ml and peaked at 21.3 ng/ml on day 7. This patient reached AKI by KDIGObin on day 10 and was thus considered a true positive. In the second case, baseline UrNGAL was 13.90 ng/ml. He reached AKI diagnosis by KDIGObin on day 8. UrNGAL levels varied from 16.58 ng/ml on day 1 to 3.58 ng/ml on day 8; peak UrNGAL level for this patient was 19.63 ng/ml on day 13. Since this was only 1.4x higher than the baseline value, we considered this a false negative case.

We tested whether analyzing UrNGAL levels as a % change from baseline values would influence the diagnostic performance of this biomarker. For these analyses, we chose an UrNGAL cutoff of ≥ 3x higher than baseline as the criterion for AKI. As illustrated in Table 2.4b, using this cutoff and SCr KDIGObin as the gold standard, we found 13 true positives, 5 false positives, 4 false negatives and 2 true negatives (sensitivity 76.5%; specificity 28.6%; positive predictive value 72.2%; negative predictive value 33.3%; accuracy 62.5%). Changing the gold standard to NT0.5 resulted in a worse diagnostic performance due to an increased frequency of false positive cases (from 5 to 7).

Time to AKI in by UrNGAL criteria

Using a cutoff of UrNGAL ≥ 2.54 ng/ml, 14 cases of AKI were detected at a mean of 5.2 ± 4.1 days. However, 3 of theses cases were considered false positive by KDIGObin criterion. In 11 cases, the diagnosis of AKI was concordant between UrNGAL and SCr (KDIGO bin) criteria. In these 11 concordant cases, AKI was detected, on average, 1.7 days earlier by UrNGAL when compared to SCr criteria (5.2 ± 3.8 versus 6.9 ± 3.5 days, UrNGAL and SCr criteria, respectively; p = 0.06). UrNGAL detected AKI earlier in 5 cases, whereas SCr prevailed in 2 cases; in the remaining 4, the diagnosis of AKI was made on the same day by both methods.

Similar analyses were performed using a cutoff of UrNGAL ≥ 3x baseline; 18 cases of AKI were detected at a mean of 3.1 ± 2.4 days. However, 5 of these cases were considered false positive by KDIGObin criterion. In 13 cases, the diagnosis was concordant between UrNGAL (≥ 3x baseline) and SCr (KDIGObin) criteria. In these 13 concordant cases, AKI was detected, on average, 3.2 days earlier by UrNGAL when compared to SCr criteria (3.7 ± 2.5 versus 6.9 ± 3.3 days, UrNGAL and SCr criteria, respectively; p = 0.001).

Figure 2.3b shows the distribution of the variable *time to AKI by UrNGAL minus time to AKI by SCr* in the 13 concordant cases. UrNGAL detected AKI earlier in 11 cases, whereas SCr prevailed in 1 case; in the remaining 1, the diagnosis of AKI was made on the same day by both methods.

DISCUSSION

We have prospectively measured daily SCr and UrNGAL in 24 non-septic, hemodynamically stable patients, treated with AmB. Our data suggests that UrNGAL has the potential to detect AmB-induced AKI earlier than the most sensitive SCr-based criteria (KDIGO). To our knowledge, this is the first study to evaluate the role of UrNGAL in the early diagnosis of AmB-induced AKI.

Since the development of RIFLE (8) and AKIN (9) criteria and publication of the recent KDIGO guidelines (46), the SCr cutoffs to define AKI are well establised. On the other hand there is still no consensus on how UrNGAL levels should be reported or which cutoffs should be used to define AKI. Several authors report UrNGAL levels as absolute ng/ml while others normalize UrNGAL for urine creatinine excretion (μg/g urine creatinine); less often, UrNGAL levels have been reported as % change from baseline (42). In our analyses, normalization for urine creatinine increased mean values but worsened the diagnostic performance of UrNGAL due to increased data dispersion. On the other hand, normalization for baseline levels did offer the advantage of equalizing all baseline levels and facilitating the establishment of a cutoff value (such as 3x increase over baseline). Nevertheless, since all subsequent values are dependent upon the baseline value, this approach is disadvantageous when the baseline value is a significant outlier.

In studies of AKI post cardiac surgery, UrNGAL starts to rise as early as 1-2 hours after cardiopulmonary bypass. In studies of contrast-induced nephropathy, UrNGAL has been measured anywhere from 2-24 hours after

contrast administration. The best timing of urine collection in studies of antimicrobial agents-related nephrotoxicity is not known. Gaspari et al. measured UrNGAL at 1 and 4 h and 1, 2, 3, 7 and 15 days after cisplatin administration and found that UrNGAL levels started to rise only after the first day (42). Considering their findings, we chose to measure UrNGAL at baseline and daily after initiation of AmB.

When looking at the entire group, UrNGAL on day 5 was associated with an AUC of 0.68 (95% CI 0.41 to 0.95) for the diagnosis of AKI (by KDIGObin). A level of UrNGAL ≥ 0.37 ng/ml on day 5 was associated with a sensitivity of 81%, specificity of 50% and accuracy of 73% in detecting AKI. In individual case analyses, we chose an UrNGAL cutoff of ≥ 2.54 ng/ml as the criteria for AKI. In these analyses, UrNGAL was associated with a sensitivity 64.7%; specificity 57.1%; positive predictive value 78.6%; negative predictive value 40.0%; and accuracy 62.5%. This diagnostic performance is somewhat inferior to that of studies of UrNGAL in other settings. In 2008, Coca et al. (47) systematically reviewed the literature and encountered four studies of good quality that investigated the role of UrNGAL as an early biomarker of AKI. Two of these studies were in the setting of cardiac surgery (16, 19), one in critically ill children (48) and one post-renal transplant (49). The sensitivity of UrNGAL for early detection of AKI varied from 73% (19) to 100% (16), specificity from 72% (48) to 98% (16), and AUC from 78% (19) to 99.8% (16). Only one of these studies looked at the ability of UrNGAL to predict AKI severity and did not find a strong association (48). Similarly, we did not find an association between AKI severity and UrNGAL levels in the present study.

UrNGAL levels tended to be higher in users of deoxycholate than in users of liposomal AmB, even when controlling for AKI status. When we analyzed subgroups of AmB preparation, we found a better diagnostic performance of UrNGAL for the detection of AKI in patients receiving deoxycholate than liposomal AmB. Using KDIGObin definition as gold standard, the AUC for UrNGAL on day 5 was 0.89 (95% CI 0.67 to 1.00); an UrNGAL level ≥ 0.23 ng/ml was associated with a sensitivity of 88.9%, sensitivity of 100% and accuracy of 90.9%. Using NT0.5 criterion, an UrNGAL on day 5 ≥ 0.23 ng/ml resulted in a perfect fit (AUC 1.0).

In 2009, Haase and coworkers also conducted a systematic review and metanalysis of NGAL for the diagnosis of AKI (14). When looking at individual studies, the authors found that UrNGAL cutoffs used for prediction of AKI varied widely, differing more than 50 times from the lowest (48) to the highest (50) reported values. In analyses of the pooled diagnostic and prognostic accuracy of NGAL, these authors found cutoffs that were almost 3 times higher in studies of cardiac surgery than in studies of contrast-induced nephropathy. This suggests that the context in which the renal injury occurs might have an impact on UrNGAL levels. The UrNGAL levels that we found in our study are similar to those reported Gaspari in recipients of cisplatin (42) and by Zappitelli et al. in critically ill children (48) but much lower than those encountered in all studies of cardiac surgery. There are no similar studies with AmB for comparison. Perhaps the less intense renal injury caused by drug nephrotoxicity was responsible for the overall low levels of UrNGAL. The

lower UrNGAL levels found in recipients of liposomal than deoxycholate AmB likely reflects the better safety profile of the liposomal preparation.

Our study has certain limitations. Since we measured UrNGAL approximately 24 hours after each dose of AmB, we cannot rule out an early UrNGAL peak. Although we were able to detect a significant difference in mean time to AKI by UrNGAL versus SCr criteria, we might have been underpowered for other comparisons, especially when looking at subgroups of AmB preparation. Moreover, our time to AKI analyses considered only paired matches of concordant cases (true positives), which is not a real world scenario. Similar to any study of urine biomarkers, the use of SCr as gold standard is in itself a limitation. Could UrNGAL elevation in patients without AKI by SCr criteria (false positive) represent "subclinical AKI"? Some experts currently recommend that, in the right clinical setting, renal tubular injury biomarkers may be used to diagnose AKI even in the absence of elevations in SCr or reductions in urine output as required by RIFLE and AKIN criteria (51). On the other hand, SCr elevations with low UrNGAL levels (false negative) could be due to a pre-renal state without overt tubular injury. Indeed, a significant component of AmB nephrotoxicity is due to direct renal vasoconstriction (52–54). Finally, recent data suggests that pyuria is an important potential confounder when measuring UrNGAL (55). We did not have urinalysis in all patients, but our sample was comprised mostly of young men, in whom urinary tract infections would be uncommon. Additionally, no patients complained of urinary symptoms or had fever or leucocytosis.

In summary, we found that UrNGAL was able to significantly shorten the time to detection of AmB-induced AKI, even when compared to the most sensitive SCr-based criteria. The diagnostic performance of UrNGAL against SCr-based criteria was moderate when looking at the entire group but excellent in the deoxycholate subgroup. Finally, UrNGAL levels were higher in recipients of deoxycholate than liposomal AmB.

Future studies should be conducted to evaluate if a UrNGAL-oriented treatment strategy will result in a reduction in the incidence of AmB-induced AKI (as defined by SCr-based criteria), as well as in hospital stay and costs. When facing a significant elevation in UrNGAL prior to an elevation in SCr, the physician could institute one (or a combination) of several measures, such as switch from a deoxycholate to a liposomal preparation, change the treatment regimen (reduce the dose, switch to alternate days or temporarily discontinue AmB), or try volume expansion with intravenous 0.9% sodium chloride. We believe that our findings with UrNGAL in AmB-induced AKI in leishmaniasis patients could serve as a basis for the investigation of this and other urine biomarkers in the early detection of drug nephrotoxicity in various clinical settings.

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